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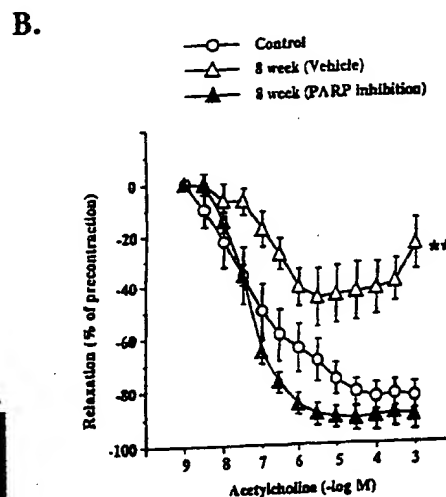
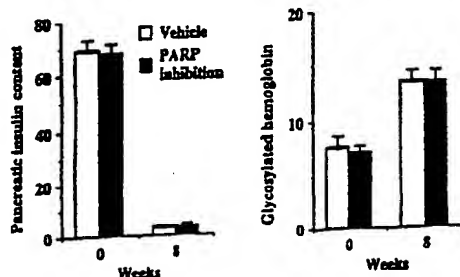
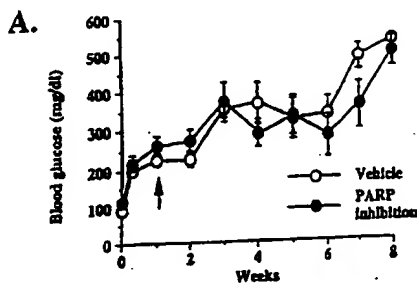
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(54) Title: NOVEL SUBSTITUTED PHENANTHRIDINONES AND METHODS OF USE THEREOF



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(57) Abstract: This invention provides a novel class of substituted 6(5H) phenanthridinone compounds. Pharmaceutical compositions, and methods of making and using the compounds, or a pharmaceutically acceptable salt, hydrate, prodrug, or mixture thereof are also described.



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ning of each regular issue of the PCT Gazette.*

NOVEL SUBSTITUTED PHENANTHRIDINONES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

The invention relates to substituted phenanthridinones.

5

BACKGROUND OF THE INVENTION

Inflammation disorders, such as arthritis, colitis, and autoimmune diabetes typically manifest themselves as disorders distinct from those associated with reperfusion injury, *e.g.*, stroke and heart attack, and can clinically manifest themselves as different entities. However, there can be common underlying mechanisms between these two types of disorders. In particular, inflammation and reperfusion injury can induce proinflammatory cytokine and chemokine synthesis. Induction of pro-inflammatory cytokines can, in turn, result in production of cytotoxic free radicals such as nitric oxide (NO) and superoxide. NO and superoxide can react to form peroxynitrite (ONOO⁻). Szabó *et al.*, Shock 6:79-88, 1996.

The peroxynitrite-induced cell necrosis observed in inflammation and reperfusion injury involves, in significant part, the activation of the nuclear enzyme poly (ADP-ribose) synthetase (PARS). Activation of PARS is thought to be an important step in the cell-mediated death observed in inflammation and reperfusion injury. Szabó *et al.*, Trends Pharmacol. Sci. 19: 287-98, 1998.

SUMMARY OF THE INVENTION

20 The invention is based in part on the discovery of novel substituted 6(5H)phenanthridinone derivatives and their unexpected effects in inhibiting inflammation, in treating reperfusion injuries, in reducing the severity of diabetes, in enhancing the function of a transplanted organ and for the treatment of symptoms associated with neurodegenerative diseases.

25 Accordingly, in one aspect the invention provides novel substituted 6(5H)phenanthridinone derivatives falling within Formula I, as set forth in the Detailed Description of the Invention, below.

Also provided is a method of treating inflammatory and reperfusion conditions in mammals by administering to a mammal in need of such treatment an effective amount of a compound according to Formula I.

5 In a further aspect, the invention also includes a method for the production of compounds of Formula I.

The substituted 6(5H)phenanthridinone compounds of the invention are potent, pharmaceutical compounds that can be used to treat a variety of conditions and diseases, typically those known to involve inflammatory mediator production and cell death.

10 The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates
15 otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All patents and publications cited in this specification are incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A and 1B are graphs and histograms showing levels of blood glucose, insulin,
20 glycosylated hemoglobin, and acetylcholine-induced relaxation of thoracic aortic rings in streptozotocin treated mice treated with vehicle or the PARS inhibitor PJ34.

FIG. 2A is a histograms showing PARP activity over time in wild-type and PARS-deficient cells activity over time, and in cells exposed to low or high concentrations of glucose in endothelial cells from PARS.

25 FIG. 2B is a histogram showing PARS activity in control cells, cells exposed to high concentrations of glucose (30 mM), and cells exposed to high concentrations of glucose and PJ34.

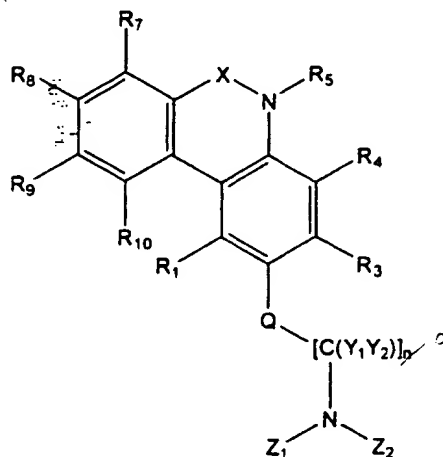
FIG. 2C is an illustration of a proposed scheme for the mechanism of PARP activation in diabetic blood vessels.

FIG. 2D is a graph showing the percentage relaxation of precontraction of endothelial cells in wild-type or PARS-deficient cells in varying concentrations of acetylcholine.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel class of 2-substituted 6(5H)phenanthridinone compounds falling within the Formula I, as set forth below:

I



where

X is C=O, C=S, SO₂, C=NH, C=NR₆; or C-Cl;

10 Q is NHCO, O, CO, OCO₂, OCO, OCONH, NR₂, NHCO₂, S, SO₂, CS, or SO;

R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, are, independently, hydrogen or lower alkyl; halogen, nitro, amino, alkylamino, carboxy, or ester;

15 Y₁ and Y₂ are, independently: hydrogen, halo, alkylhalo, hydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₃-C₈ carbocyclic, aryl, alkylamino, amino, carboxy, ester, arylalkyl, or nitro;

n is 0 to 10; and

20 Z₁ and Z₂ are, independently: hydrogen, alkylhalo, alkylhydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₂-C₁₀ straight or branched chain alkynyl group, aryl, benzyl, alkylamino, alkylcarboxy, alkylester, arylalkyl, or Z₁ or Z₂ taken together form a fused ring, wherein the ring has 4-8 ring members.

In addition to the compounds of Formula I, the invention also provides a pharmaceutically acceptable base or acid addition salt, hydrate, ester, solvate, prodrug, metabolite, stereoisomer, or mixture thereof. The invention also includes pharmaceutical formulations comprising a compound of Formula I in association with a pharmaceutically acceptable carrier, diluent, or excipient.

Also included are pharmaceutically acceptable derivatives of the foregoing compounds, where the phrase "pharmaceutically acceptable derivative" denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof, preferably one which is capable of eliciting a desired response. Pharmaceutically acceptable derivatives thus include, among others, pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety which is susceptible to removal in vivo yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester which is cleaved in vivo to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. One technique for linking a prodrug to a compound of the present invention is described generally in Niemi *et al.*, J. Med. Chem. 42:5053-5058, 1999.

With respect to the compounds of the present invention, a named R group will generally have the structure which is recognized in the art as corresponding to R groups having that name. For the purposes of illustration, representative R groups as enumerated in the specification and claims of the present application are defined herein. These definitions are intended to supplement and illustrate, not preclude, the definitions known to those of skill in the art. A comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry*; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

As used herein:

The term "independently selected" is used herein to indicate that the R groups can be identical or different. As used herein, the definition of each expression, *e.g.* alkyl, n, etc., when it

occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C1-C30 for straight chain, C3-C30 for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure. Typical alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tertiary butyl, pentyl, hexyl and the like.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxy, a phosphoryl, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF₃, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF₃, -CN, and the like.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (*e.g.*, an aromatic or heteroaromatic group). Exemplary aralkyl groups include, but are not limited to, benzyl and more generally (CH₂)_nPh, where Ph is phenyl or substituted phenyl, and n is 1, 2, or 3.

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one carbon-carbon double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means
5 an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The term "aryl" as used herein includes 5-, 6- and 7-membered single-ring aromatic
10 groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen,
15 azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -CF₃, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused
20 rings") wherein at least one of the rings is aromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The terms ortho, meta and para apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

25 The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine,
30 indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine.

furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, 5 nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, -CN, or the like.

"Alkylhalo" refers an alkyl group containing a halogen substituent; "alkylhydroxy" refers to an alkyl group having a hydroxyl substituent; "alkylamino" refers to an alkyl group having an amino substituent; "alkylester" refers to an alkyl group having an ester functionality appended 10 thereto; "alkylcarboxy" refers to an alkyl group having a carboxyl functionality appended thereto. "Halo" refers to chloro, fluoro, bromo or iodo.

"Alkoxy" refers to the radical --O-alkyl. Typical alkoxy radicals are methoxy, ethoxy, propoxy, butoxy and pentoxy and the like.

15 "Cycloalkyl" refers to saturated monocyclic hydrocarbon radical containing 3-8 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and the like.

The carrier must be "acceptable" in the sense of being compatible with the active ingredient of the formulation and not deleterious to the subject to be treated. Preferably, the 20 carrier is also capable of stabilizing the compound or composition.

Whenever the term "alkyl" or its prefix root appears in a name of a substituent (*e.g.* aralkoxyaryloxy) it shall be interpreted as including those limitations given above for "alkyl".

"Subject" shall mean a human or animal (*e.g.* rat, mouse, cow, pig, horse, sheep, monkey, cat, dog, goat etc.).

25 It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

As used herein, the term "substituted" is contemplated to include all permissible 30 substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and

nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., PARS inhibiting agents), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

In some embodiments, X is C=O.

In some embodiments, one or more R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, Y₁, or Y₂ are hydrogen.

In some embodiments, Q is NHCO.

In some embodiments, n is 1.

In some embodiments, one or both of Z₁ and Z₂ are methyl groups (PJ-34).

In some embodiments, one or both of Z₁ and Z₂ are ethyl groups (PJ-44).

5 In some embodiments, Z₁ is a methyl group and Z₂ is a benzyl group (PJ-45).

In some embodiments, N, Z₁ and Z₂ taken together, form piperidine, piperazine, N-alkylated or alkylcarbonylated piperazine, pyrrole, imidazole, indole, or other C₂ to C₁₀ branched or cyclic or cycloalkenyl amines.

10 In some embodiments, Z₁, N, and Z₂ taken together form a fused ring having six ring members.

In some embodiments, Z₁ and Z₂ taken together form -CH₂-CH₂-CH₂-CH₂-CH₂- (PJ-36).

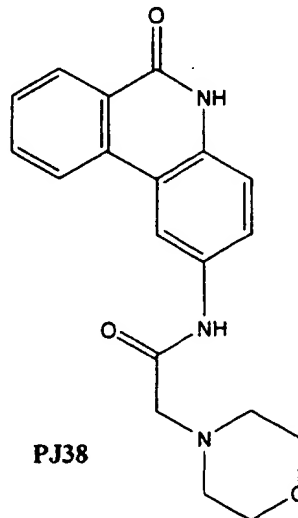
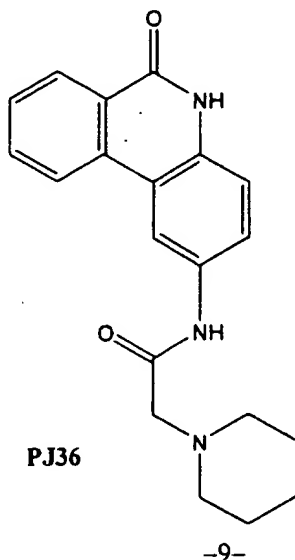
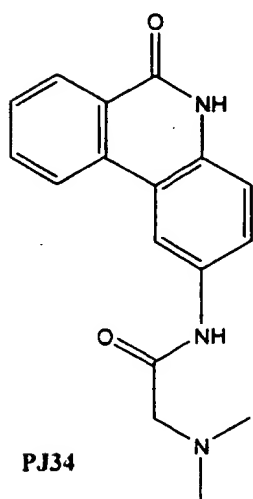
In some embodiments, at least one of the ring members is oxygen.

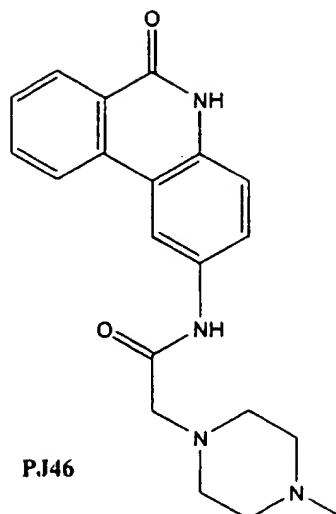
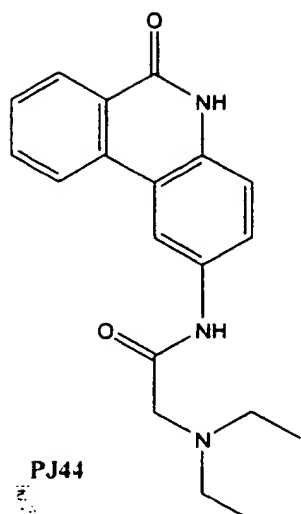
In some embodiments, Z₁ and Z₂ taken together form -CH₂-CH₂-O-CH₂-CH₂- (PJ-38).

In some embodiments, two of the ring members are nitrogen atoms.

15 In some embodiments, Z₁ and Z₂ taken together form -CH₂-CH₂-N(CH₃)-CH₂-CH₂- (PJ-46).

In particularly preferred embodiments, the compounds have the structures represented by PJ 34, 36, 38, 44 and 46, as shown below.





Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid to produce "pharmaceutically-acceptable acid addition salts" of the compounds described herein. These compounds retain the biological effectiveness and properties of the free bases. Representative of such salts are the water-soluble and water-insoluble salts, such as the acetate, amsonate (4,4-diaminostilbene-2, 2'-disulfonate), benzenesulfonate, benzonate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium edetate, camsylate, carbonate, chloride, citrate, clavulinate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexafluorophosphate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, 3-hydroxy-2-naphthoate, oleate, oxalate, palmitate, pamoate (1,1-methylene-bis-2-hydroxy-3-naphthoate, embonate), pantothenate, phosphate/diphosphate, picrate, polygalacturonate, propionate, p-toluenesulfonate, salicylate, stearate, subacetate, succinate, sulfate, sulfosalicylate, suramate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate salts.

Methods of using substituted 6(5H)phenanthridinone derivatives

The invention also includes a method of inhibiting poly(ADP)-ribose synthase activity (PARS) in a cell. This enzyme, which is also known as poly(ADP-ribose) polymerase (PARP,

EC 2.4.99), and ADP-ribosyltransferase (ADPRT, EC 2.4.2.30), is a nuclear enzyme that catalyzes a transfer of the ADP ribose moiety of NAD⁺ to an acceptor protein.

The method includes contacting the cell with a compound of Formula I in an amount sufficient to inhibit poly (ADP)-ribose-synthase in the cell. In general, any cell having, or
5 capable of having, PARS activity, can be used. The can be provided in any form so long as it is accessible to the compound. For example, the cell can be provided *in vitro*, *ex vivo*, or *in vivo*. PARS activity can be measured using any method known in the art, *e.g.*, methods as described in Banasik *et al.*, J. Biol. Chem. 267:1569-75, 1991.

Also provided in the invention is a method of inhibiting, preventing, or treating
10 inflammation in a subject. The inflammation can be associated, *e.g.*, with an inflammatory disease. Inflammatory diseases refer to diseases or conditions where there is an inflammation of the body tissue. These include local inflammatory responses and systemic inflammation. Examples of such diseases and conditions include: transplant rejection; chronic inflammatory disorders of the joints, including arthritis, rheumatoid arthritis, osteoarthritis and bone diseases
15 associated with increased bone resorption; inflammatory bowel diseases such as ileitis, ulcerative colitis, Barrett's syndrome, and Crohn's disease; inflammatory lung disorders such as asthma, adult respiratory distress syndrome, and chronic obstructive airway disease; inflammatory disorders of the eye including corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis and endophthalmitis; chronic inflammatory disorders of the gum, including
20 gingivitis and periodontitis; tuberculosis; leprosy; inflammatory diseases of the kidney including uremic complications, glomerulonephritis and nephrosis; inflammatory disorders of the skin including sclerodermitis, psoriasis and eczema; inflammatory diseases of the central nervous system, including chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis,
25 encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and viral or autoimmune encephalitis; autoimmune diseases, immune-complex vasculitis, systemic lupus and erythematodes; systemic lupus erythematosus (SLE); and inflammatory diseases of the heart such as cardiomyopathy, ischemic heart disease hypercholesterolemia, atherosclerosis; as well as various other diseases with significant inflammatory components, including
30 preeclampsia; chronic liver failure, brain and spinal cord trauma, and cancer. There may also be a systemic inflammation of the body, exemplified by gram-positive or gram negative shock, hemorrhagic or anaphylactic shock, or shock induced by cancer chemotherapy in response to

pro-inflammatory cytokines. *e.g.*, shock associated with pro-inflammatory cytokines. Such shock can be induced. *e.g.*, by a chemotherapeutic agent used cancer chemotherapy.

The invention also includes a method of treating, preventing, or otherwise inhibiting reperfusion injury in a subject in need of treatment, prevention, or inhibition thereof. The method includes administering a compound of Formula I in an amount sufficient to inhibit reperfusion injury in the subject. Reperfusion refers to the process whereby blood flow in the blood vessels is resumed after blood flow has been interrupted, such as occurs following constriction or obstruction of the vessel. Reperfusion is typically associated with ischemia and may result following a naturally occurring episode, such as a myocardial infarction or stroke, or during a surgical procedure where blood flow in vessels is purposely or unintentionally blocked off.

Also included in the invention is a method for treating or preventing diabetes in a subject. In another aspect, the invention provides a method for treating complications, *e.g.*, vascular complications, associated with diabetes by administering a PARS inhibitor.

Patients with advanced Type I or Type II diabetes mellitus frequently suffer from associated retinopathy and peripheral vascular disease. Diabetic vasculopathy also predisposes a subject for accelerated atherosclerosis and hypertension, and is a significant risk factor for end-stage renal disease, coronary atherosclerosis, and cerebrovascular disease. Tooke *et al.*, Diabet. Med. 16: 710-5, 1999; Keen *et al.*, Diabetes Metab. Res. Rev. 15: 186-96, 1999. Endothelial injury and dysfunction is a critical constituent of diabetic vasculopathy. Tooke *et al.*, Diabet. Med. 16: 710-5, 1999; Keen *et al.*, Diabetes Metab. Res. Rev. 15: 186-96, 1999. Oxygen-derived free radicals and oxidants play an important role in the pathogenesis of endothelial dysfunction. Rodriguez-Manas L *et al.*, Br. J. Pharmacol. 123: 1495-502, 1998; Graier *et al.*, Diabetes Res. Clin. Pract. 45:153-60, 1999; Nishikawa *et al.*, Nature 404: 787-90, 2000. Oxidant mediated cell death and dysfunction have recently been linked to activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP). Szabo and Dawson, Trends Pharmacol. Sci. 19: 287-98, 1998; Pieper *et al.*, Trends Pharmacol. Sci. 20: 171-81, 1999.

While not wishing to be bound by theory, it is believed that activation of PARP is an important factor in the pathogenesis of endothelial dysfunction in diabetes. Destruction of functional islet cells with streptozotocin in mice induces chronic hyperglycemia, intravascular production of oxidants, activation of PARP in the endothelium, and a loss of endothelial function. Treatment with a novel potent phenanthridinone PARS inhibitor, starting after the time

of islet destruction, was observed to maintain normal vascular responsiveness. This responsiveness was maintained despite the persistence of severe hyperglycemia. Human and murine endothelial cells incubated in high glucose medium exhibited prolonged PARP activation, which was due to the intracellular production of reactive nitrogen and oxygen species.

5 Vascular rings from PARP^{-/-} mice incubated in high glucose medium showed a loss of endothelial function, an effect prevented by pharmacological inhibition of PARP and did not develop in the vascular rings from PARP^{+/+} mice. Thus, PARS activation is implicated in the pathogenesis of diabetic endothelial dysfunction.

Diabetic vascular dysfunction is a major clinical problem, which predisposes to a variety of cardiovascular diseases. Hyperglycemic episodes are thought to play a major role in the development of the endothelial dysfunction, which represents a major component of diabetic vasculopathy. Tooke *et al.*, Diabet. Med. 16: 710-5, 1999; Keen *et al.*, Diabetes Metab. Res. Rev. 15: 186-96, 1999; Rodriguez-Manas L *et al.*, Br. J. Pharmacol. 123: 1495-502, 1998; Graier *et al.*, Diabetes Res. Clin. Pract. 45:153-60, 1999; Nishikawa *et al.*, Nature 404: 787-90, 15 2000. The reduced production of nitric oxide from the vascular endothelial cells in diabetes predisposes to platelet and neutrophil deposition, vasospasm, hypertension, stroke and myocardial infarction, as well as accelerated atherosclerosis. The generation of intracellular oxidants within the mitochondria of the diabetic endothelium, and in the vicinity of endothelium, plays a key role in the loss of endothelial function in diabetes. Nishikawa *et al.*, Nature 404: 20 787-90, 2000.

Accordingly, the invention includes a method of treating or preventing diabetes by administering to a subject in need thereof an amount of an inhibitor of a poly (ADP-Ribose) polymerase (PARS) sufficient to lessen or prevent the symptoms of diabetes in the subject. The compound preferably acts by inhibiting PARS in the subject. In some embodiments, the PARS 25 inhibitor is a compound of Formula I, as described above. In other embodiments, the inhibitor can be, *e.g.*, benzamide, 3-aminobenzamide, nicotinamide, 4-aminobenzamide, and 1,5-dihydroxy-isoquinoline. In some embodiments, administering the inhibitor does not increase insulin levels in the subject.

The method can be used to treat a subject that has, *e.g.*, Type I or Type II diabetes, and/or 30 any conditions or diseases associated with diabetes. The conditions or diseases can include, *e.g.*, the diseases related to cardiovascular complications of Type I or Type II diabetes. Examples of such disease include, *e.g.*, retinopathy, nephropathy, peripheral vascular disease, vascular aneurysm, limb ischemia, ischemia-related limb gangrene, ulcers, including diabetic foot ulcer.

and skin lesions, claudication, autonomic nervous system dysfunction, peripheral neuropathy, organic impotence, accelerated atherosclerosis, cardiomyopathy, myocardial dysfunction, ischemia and infarction, cerebrovascular disease.

The invention also provides a method of increasing or maintaining vascular
5 responsiveness, *e.g.*, appropriate contractile and endothelium-dependent relaxant responsiveness, in an endothelial cell. In some embodiments, the endothelial cell is provided in a blood vessel. The cell can be provided, *e.g.*, *in vitro*, *ex vivo*, or *in vivo*. For example, the method can include administering to a subject in need thereof an amount of an inhibitor of PARS in an amount
10 sufficient to increase or maintain vascular responsiveness, *e.g.*, by inhibiting activation or activity of PARS in the subject. The inhibitor can be, *e.g.*, a PARS inhibitor according to Formula I, as described above. Alternatively, or in addition, the inhibitor can be, *e.g.*, benzamide, 3-aminobenzamide, nicotinamide, 4-aminobenzamide, and 1,5, dihydroxy-isoquinoline.

Additionally, the invention provides a method of enhancing the function of a transplanted
15 organ in a subject, *e.g.*, a human subject. In one embodiment, prior to transplanting an organ into a patient, the organ is contacted with a PARS inhibitor in an amount sufficient to inhibit PARS in the organ. Additionally, the inhibitors of the invention can be used to prolong the function of a transplanted organ in a subject. In some aspects, the method can include administering to the subject a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount sufficient to inhibit
20 PARS in said subject before, during, or after the transplant operation.

For example, the method includes administering to a subject a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount sufficient to enhance the function of the transplanted organ in the subject. The inhibitor can be, *e.g.*, a PARS inhibitor according to Formula I, as described above. Alternatively, or in addition, the inhibitor can be, *e.g.*, benzamide, 3-
25 aminobenzamide, nicotinamide, 4-aminobenzamide, and 1,5, dihydroxy-isoquinoline. In some embodiments, the organ is contacted with the inhibitor prior to transplantation. In other embodiments, the inhibitor is administered to the subject at the time of transplantation of the organ.

The method is useful for enhancing the function of a variety of transplanted organs,
30 including, but not limited to, heart, kidney, lung, liver, retina, pancreatic islet, blood vessel, skin, and bone.

In some embodiments, a PARS inhibitor is used in combination with an immuno-suppressant. Suitable immuno-suppressants include, *e.g.*, cyclosporine, tacrolimus, mycophenolate mofetil, corticosteroids, azathioprine, and mixtures thereof.

5 In addition to, or instead of an immuno-suppressant, the PARS inhibitor may be used with an organ preservative solution, or a mixture of organ preservation and cardioplegic solutions.

Generally, current donor organ preservation protocols utilize hypothermic (below 20 °C and typically at about 4 °C) arrest and storage in a chemical perfusate for maintaining the heart (non-beating) or other organ (non-functioning) for up to four hours. However, these protocols
10 utilize a variety of crystalloid-based cardioplegic solutions that do not completely protect the donor heart from myocardial damage resulting from ischemia and reperfusion injuries. In addition to myocardial damage, ischemia, reperfusion and/or increased potassium concentrations may also cause coronary vascular endothelial and smooth muscle injury leading to coronary vasomotor dysfunction, which is believed to be the leading cause of late organ failure.
15 Additionally, significant medical advantages would be achieved if organs for transplantation could be stored and preserved for 2-3 days and longer. Longer storage times provide additional time for histocompatibility testing of the donor and recipient, organ viability testing and provides additional time for preoperative decisions and preparations.

Numerous preservative solutions have been developed and used to preserve major organs
20 while they are in cold storage prior to their transplantation. Optimal preservation solutions include those which minimize ischemic and/or reperfusion injuries, minimize cell swelling and edema; prevent intracellular acidosis; and provide substrates for regeneration of high-energy phosphate compounds and ATP during reperfusion. The most commonly used preservation solutions for transplantation include crystalloid cardioplegia consisting of isotonic or slightly
25 hypertonic saline supplemented with glucose and potassium chloride, buffering capacity is usually afforded by the addition of sodium bicarbonate. In addition, some solutions contain small amounts of magnesium or calcium, glucose, ATP and creatine phosphate, while others contain pharmacologic agents such as mannitol, insulin, procaine or calcium channel blockers. See, *e.g.*, U.S. Patent 5,066,578, U.S. Patent 6,046,046, U.S. Patent 6,037,116. See also, U.S.
30 Patent 5,552,267 for disclosure on preservation solutions containing a phosphodiesterase inhibitor.

Organ preservative solutions include, without limitation, University of Wisconsin (UW) or Belzer's solution (U.S. Patent 4,798,824) which is marketed by DuPont as VIASPANTM solution; Marshall's preservation fluid; histidine-tryptophan-alpha-ketoglutarate solution (Kallerhoff *et al.*, Transplantation 39:485-489, 1985); Phosphate buffered extracellular (Ep4) solution (Handa *et al.*, J. Exp. Med. 159:205, 1988); St. Thomas solution; Columbia solution (U.S. Patent 5,552,267); CMH solution (Arita *et al.*, Transplant Proc. 5:336, 1992); and Stanford solution (Swanson *et al.*, J of Heart Transplant. 7:456-467, 1988).

Other widely used preservative solutions are the Collins (Collins, The Lancet 1219-1222, 1969) and the Euro-Collins (Squifflet *et al.*, Transplant. Proc. 13:693-696, 1981) solutions. These solutions resemble intracellular fluid and contain glucose as an osmotic agent. In addition to glucose, high osmolality preservative solutions have been prepared using raffinose and lactobionate as in the UW preservative solution (Ploeg *et al.*, Transplant. Proc., 20 (suppl 1) 1:935-938, 1988; U.S. Patent 4,798,824), mannitol in the Sacks solution (Sacks, The Lancet 1:1024-1028, 1973), sucrose in the phosphate buffered sucrose (PBS) preservative solution (Lam *et al.*, Transplantation 47:767-771, 1989) and the histidine buffered HTK solution of Bretschneider (Kallerhoff *et al.*, Transplantation 39:485-489, 1985). Hypertonic citrate preservative solutions are also known. Ross *et al.*, Transplantation 21:498-501, 1976. The effectiveness of these solutions as preservative solutions for organs appears to be related to the specific osmotic agent which is used. Coffey and Andrews, Transplantation 35:136-143, 1983. Preservative solutions are also known which contain synthetic hydroxyethyl starch (HES) as an osmotic colloid. See, e.g., U.S. Pat. Nos. 4,879,283, 4,798,824, and 5,082,831.

Preservative solutions are also known for preserving corneas for transplantation. Corneal preservative solutions are designed to prevent endothelial cell damage. Corneal preservative solutions containing glucose or dextran are known. Kaufman *et al.*, Arch. Ophthalmol 109:864-868, 1991; McCarey and Kaufman Invest. Ophthalmol. 13: 165 and 859, 1974. The corneal preservative solutions known as OPTISOLTM, DEXSOLTM and MKTM contain DEXTRAN 40 (average molecular weight=40,000 daltons) as an osmotic agent. U.S. Patent 5,306,711 discloses preservative solutions containing dextran having an average molecular weight of 10,000 daltons or less. U.S. Patent 5,145,771 describes a storage solution for preserving organs at temperatures between 0 and 37 °C. U.S. Patent 5,693,462 discloses organ preservation solutions containing compounds such as amiloride and derivatives thereof which are useful for inhibiting Na⁺/H⁺ exchange. The preservation solutions described therein include a balanced isotonic solution of sodium, potassium, calcium, and magnesium ions and bicarbonate in a physiologically

acceptable amount, and an amiloride-containing compound. The amiloride-containing compound may be amiloride, hexamethylene amiloride, dimethyl amiloride, ethyl isopropyl amiloride or methyl isobutyl amiloride. The preserving solutions may also include other components such as EDTA, a small amount of adenosine, and at least one antioxidant, such as, 5 dimethyl thiourea (DMTU), catalase as a hydrogen peroxide scavenger and apoferritin to decrease iron content within the preservation solution. In addition, the preservation solutions optionally may include hormones, such as insulin and prostaglandin and antibiotics.

Also provided by the invention is a method of treating symptoms associated with a neurodegenerative disorder in a subject. The method includes administering to the subject PARS 10 inhibitor in an amount to alleviate symptoms of the neurodegenerative disorder in the subject. The neurodegenerative disorder can be, *e.g.*, multiple sclerosis ("MS"), AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and encephalitis.

The invention also includes methods of inhibiting the development of symptoms 15 associated with a neurological disorder, *e.g.*, multiple sclerosis, in a subject by administering to a subject at risk for the development of the symptoms a PARS inhibitor in an amount to inhibit the development of symptoms of multiple sclerosis in said subject.

In some embodiments, the PARS inhibitor is used to treat, or prevent the development of symptoms associated with MS. Multiple sclerosis (MS) is an inflammatory disease of the central 20 nervous system (CNS) white matter. The disease is characterized by demyelination, focal T cell and macrophage infiltrates, axonal injury, and loss of neurological function. Bar-Or *et al.*, Journal of Neuroimmunol. 100:252-259, 1999. The term multiple sclerosis refers to multiple areas of patchy scarring, or plaques, that result from demyelination, or the destruction of myelin, a fatty insulation covering the nerve fibers. Myelin coats lie in segments along the axons, the 25 long filaments that carry electric impulses away from a nerve cell. When the myelin sheath is destroyed during the MS disease process, signals transmitted throughout the CNS are disrupted. The nerves retain the capacity to remyelinate; however, the disease process usually outpaces these corrective actions.

Multiple sclerosis is defined as an autoimmune disease; that is, the body's immune system 30 is damaged by genetic or environmental factors (or both), causing it to attack its own tissues. In the case of MS, these tissues are the myelin covering the nerve fibers in the brain. While not wishing to be bound by theory, one mechanism advanced for the development of MS is that a

genetically damaged immune system is unable to distinguish between virus proteins and the body's own myelin and so produces antibodies that attack. When an injury or an infection occurs, the immune system mobilizes white blood cells and other factors to rid the body of any foreign proteins such as viruses.

5 The masses of blood cells that gather at the injured or infected site cause the area to become inflamed. Under normal conditions, this inflammatory process is controlled and self-limiting. However, in people with autoimmune diseases such as multiple sclerosis, the process persists and damages surrounding tissues. The inflammatory response from the immune response may also be responsible for axon destruction, a phenomenon that may account for the
10 irreversibility of MS. Alternatively, the inflammatory response may develop after the protective myelin is removed, and the axon is exposed to injury.

MS-associated symptoms include, *e.g.*, optic neuritis, fatigue, spasticity, imbalance, tremors, incontinence, constipation, sexual dysfunction, hearing loss, vertigo, facial pain, and difficulties in swallowing, and mental function.

15 MS is often studied in an animal model system termed experimental autoimmune encephalomyelitis (EAE). EAE is an inflammatory condition characterized by multifocal perivascular CNS inflammatory infiltrates that primarily include T cells and monocytes. Bar-Or *et al.*, Journal of Neuroimmunol. 100:252-259, 1999. EAE can be induced in animals by injection of immunodominant peptides from myelin proteins such as myelin basic protein
20 (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), or by transfer of CD4+ MHC class II -restricted T-cells reactive with these peptides. Mokhtarian *et al.*, Nature 309: 312-314, 1984; Zamvill *et al.*, Nature 317:355-358, 1985. The EAE models are frequently used to study the pathogenesis of MS and to test novel therapeutic strategies aimed at treating MS. As is described in the Examples, the PARS inhibitor PJ34 suppresses symptoms
25 associated with EAE in a rat model.

The PARS inhibitor used to treat, or prevent the development of symptoms associated with a neurodegenerative disorder can be, *e.g.*, a PARS inhibitor according to Formula I, as described above. Alternatively, or in addition, the inhibitor can be, *e.g.*, benzamide, 3-aminobenzamide, nicotinamide, 4-aminobenzamide, and 1,5, dihydroxy-isoquinoline.

30 A PARS inhibitor can be used in conjunction with other compounds used to treat MS or its symptoms. For example, A PARS inhibitor of the invention may be used in conjunction with

interferons, *e.g.*, interferon alpha, interferon beta. Interferon beta drugs include IFN1b and IFN1a. Other interferons include oral forms of interferon and interferon alpha n-3.

One or more PARS inhibitors can additionally be used in association with, *e.g.*, human transforming growth factor (TGF) beta-2, cyclophosphamide, glatiramer acetate (also known as Cop-1 or copolymer-1), azathioprine, corticosteroids, *e.g.*, methylprednisolone given
5 intravenously or oral prednisone, immunoglobulins, *e.g.*, intravenous immunoglobulin treatment (such as intravenous immunoglobulin treatments are monthly infusions of natural antibodies), or one or more immunosuppressant treatments. The associated immunosuppressant treatment can be a chemical or physical treatment. For example, immunosuppressant agents or procedures can
10 include total lymphoid irradiation, cladribine, mitoxantrone, sulfasalazine, cyclosporine, acyclovir, and oral bovine myelin.

The PARS inhibitors may also be used along with, *e.g.*, a vaccine against T-cells that destroy myelin; peptides that block the actions of T-cells (*e.g.*, a peptide that blocks the signals of CD-4, which is known to attack myelin); monoclonal antibodies designed to target immune
15 factors thought to be responsible for MS (*e.g.*, Hu23F2g, designed to attach to immune cells and prevent their movement across the blood-brain barrier); aminopyridines, *e.g.*, 4-aminopyridine and 3,4-diaminopyridine; drugs that inhibit tumor necrosis factors (*e.g.*, thalidomide and rolipram).

The PARS inhibitor can be used in conjunction with drugs, such as amantadine or
20 pemoline, baclofen, diazepam, dantrolene, nabilone carbamazepine and glutethimide, anticonvulsive medications such as carbamazepine, gabapentin, phenytoin, diazepam, or pimozone, or the antidepressant amitriptyline, tricyclic antidepressant, *e.g.*, amitriptyline, desipramine and imipramine, or serotonin-reuptake inhibitors (SSRIs), which include fluoxetine, sertraline, and paroxetine.

25 The subject in the above-mentioned methods can be, *e.g.*, a mammal, *e.g.*, a human, mouse, rat, dog, cat, horse, cow, pig, or non-human primate. Administration can be systemic or topical, and can be prophylactic or therapeutic.

The term "pharmacologically effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system,
30 animal or human that is being sought by a researcher or clinician.

The compounds disclosed herein can be used for the manufacture of medicaments for treating and/or preventing various diseases and disorders, for example, medicaments useful for

inhibiting poly(ADP)-ribose synthase activity; for treating or preventing local inflammation (*e.g.*, an inflammatory disorder of a joint, an inflammatory bowel disease, an inflammatory lung disorder, an inflammatory disease of the central nervous system, or an inflammatory disease of the eye) or systemic inflammation (*e.g.*, gram-positive shock, gram negative shock, hemorrhagic shock, anaphylactic shock, traumatic shock, and chemotherapeutic shock); for treating or preventing a disease or condition relating to cardiovascular complications of diabetes (*e.g.*, vascular degeneration, retinopathy, nephropathy, peripheral vascular disease, vascular aneurysm, limb ischemia, ischemia-related limb gangrene, ulcer, skin lesion, claudication, autonomic nervous system dysfunction, peripheral neuropathy, organic impotence, accelerated atherosclerosis, cardiomyopathy, myocardial dysfunction, ischemia, infarction, and cerebrovascular disease); for enhancing the function of a transplanted organ (*e.g.*, lung, liver, retina, pancreatic islet, blood vessel, skin, bone, kidney and heart); for treating symptoms associated with multiple sclerosis or other neurodegenerative disorders (*e.g.*, AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and an encephalitis); and for treating or preventing reperfusion injury (*e.g.*, myocardial infarction or stroke).

The invention also includes pharmaceutical compositions suitable for treating, inhibiting or preventing inflammation or reperfusion injury, PARS activity, cardiovascular complications, multiple sclerosis, neurodegenerative disorders, and the like, or more than one of these activities. The compositions are preferably suitable for internal use and include an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more pharmaceutically acceptable carriers. The compounds are especially useful in that they have very low, if any toxicity.

In practice, the compounds, medicaments, or their pharmaceutically acceptable salts, are administered in amounts which will be sufficient to inhibit inflammatory conditions or disease and/or prevent the development of inflammation or inflammatory disease in animals or mammals, and are used in the pharmaceutical form most suitable for such purposes.

For instance, for oral administration in the form of a tablet or capsule (*e.g.*, a gelatin capsule), the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, magnesium aluminum silicate, starch paste, gelatin, methylcellulose, sodium carboxymethylcellulose and/or

polyvinylpyrrolidone, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, silica, talcum, stearic acid, its
5 magnesium or calcium salt and/or polyethyleneglycol and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, starches, agar, alginic acid or its sodium salt, or effervescent mixtures, and the like. Diluents, include, *e.g.*, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine.

Injectable compositions are preferably aqueous isotonic solutions or suspensions, and
10 suppositories are advantageously prepared from fatty emulsions or suspensions. The compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. The compositions are prepared according to conventional mixing, granulating or coating methods,
15 respectively, and contain about 0.1 to 75%, preferably about 1 to 50%, of the active ingredient.

The compounds of the invention can also be administered in such oral dosage forms as timed release and sustained release tablets or capsules, pills, powders, granules, elixers, tinctures, suspensions, syrups and emulsions.

Liquid, particularly injectable compositions can, for example, be prepared by dissolving,
20 dispersing, etc. The active compound is dissolved in or mixed with a pharmaceutically pure solvent such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form the injectable solution or suspension. Additionally, solid forms suitable for dissolving in liquid prior to injection can be formulated. Injectable compositions are preferably aqueous isotonic solutions or suspensions. The compositions may be sterilized and/or contain
25 adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances.

The compounds of the present invention can be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, all using forms well known to
30 those of ordinary skill in the pharmaceutical arts. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions.

Parental injectable administration is generally used for subcutaneous, intramuscular or

intravenous injections and infusions. Additionally, one approach for parenteral administration employs the implantation of a slow-release or sustained-released systems, which assures that a constant level of dosage is maintained, according to U.S. Pat. No. 3,710,795, incorporated herein by reference.

5 Furthermore, preferred compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Other preferred
10 topical preparations include creams, ointments, lotions, aerosol sprays and gels, wherein the concentration of active ingredient would range from 0.1% to 15%, w/w or w/v.

For solid compositions, excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound defined above, may be also
15 formulated as suppositories using for example, polyalkylene glycols, for example, propylene glycol, as the carrier. In some embodiments, suppositories are advantageously prepared from fatty emulsions or suspensions.

The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar
20 vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines. In some embodiments, a film of lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in U.S. Pat. No. 5,262,564.

Compounds of the present invention may also be delivered by the use of monoclonal
25 antibodies as individual carriers to which the compound molecules are coupled. The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide-phenol, polyhydroxyethylaspanamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may
30 be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or

amphipathic block copolymers of hydrogels.

If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and other substances such as for example, sodium acetate, triethanolamine oleate, etc.

5 The dosage regimen utilizing the compounds is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to
10 prevent, counter or arrest the progress of the condition.

Oral dosages of the present invention, when used for the indicated effects, will range between about 0.05 to 1000 mg/day orally. The compositions are preferably provided in the form of scored tablets containing 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mg of active ingredient. Effective plasma levels of the compounds of the present
15 invention range from 0.002 mg to 50 mg per kg of body weight per day.

Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily.

If desired, the pharmaceutical compositions can be provided with an adjuvant. Adjuvants are discussed above. In some embodiments, adjuvants can be used to increase the
20 immunological response, depending on the host species, include Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Generally, animals are injected with antigen using several
25 injections in a series, preferably including at least three booster injections.

Any of the above pharmaceutical compositions may contain 0.1-99%, preferably 1-70% of the active compounds, especially compounds of Formula I as active ingredients.

Also within the invention is a method of identifying a candidate agent for treating diabetic vascular complications. The method includes contacting a PARS enzyme with a test
30 compound with the PARS enzyme within endothelial or smooth muscle cells, and determining whether the compound inhibits the activity of said enzyme. An inhibition of PARS activity in the presence of the compound indicates the test agent is a candidate agent *e.g.*, for treating

inflammation, multiple sclerosis and other neurodegenerative disorders, diabetes, for enhancing the function of transplanted organs, and/or for treating vascular dysfunction.

Synthesis of novel phenanthridinones

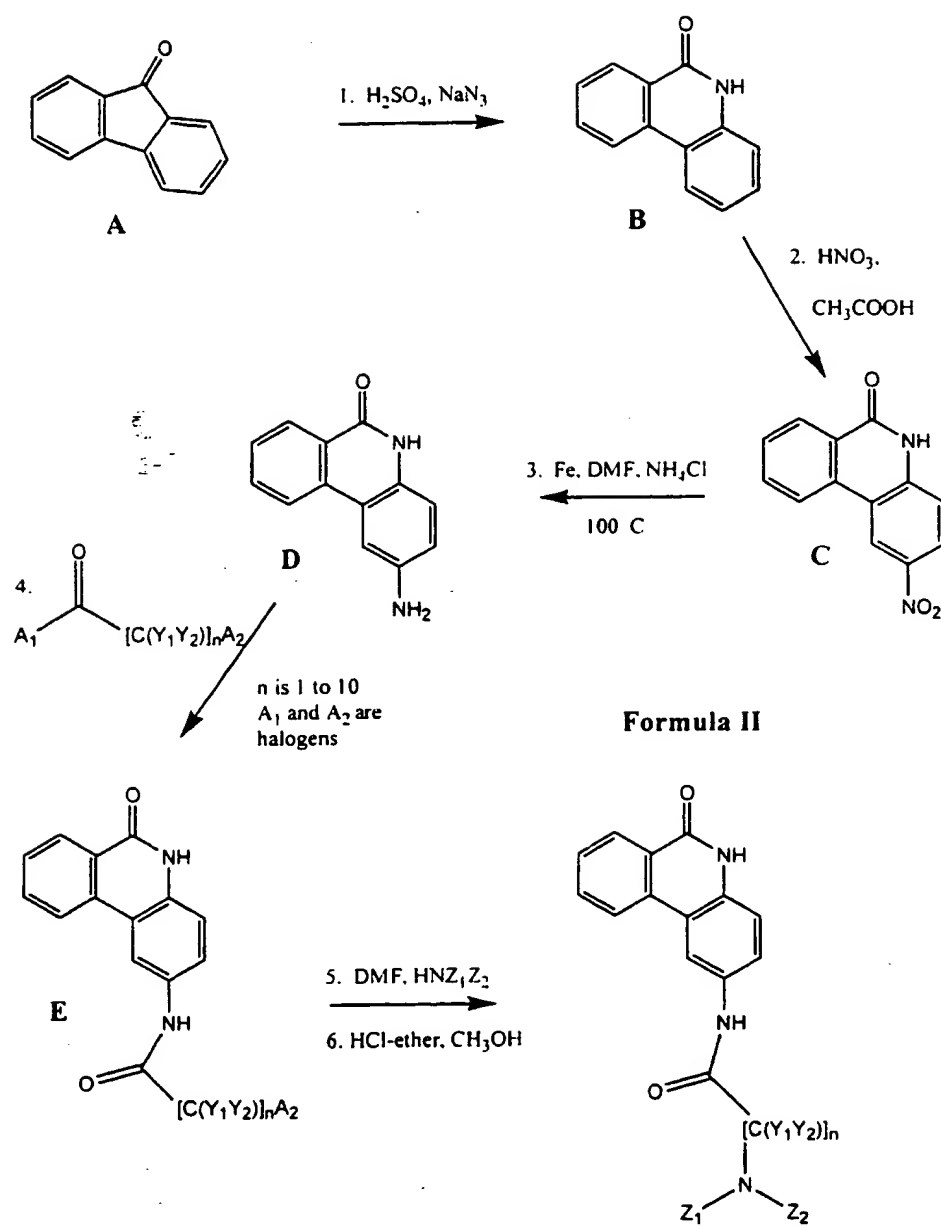
5 The substituted 6(5H) phenanthridinone compounds described herein can be prepared by methods well known in the art. Additional exemplary synthetic routes for compounds having the general structure of Formula II, which describes compounds encompassed by Formula I, are illustrated as Scheme I and Scheme II, below.

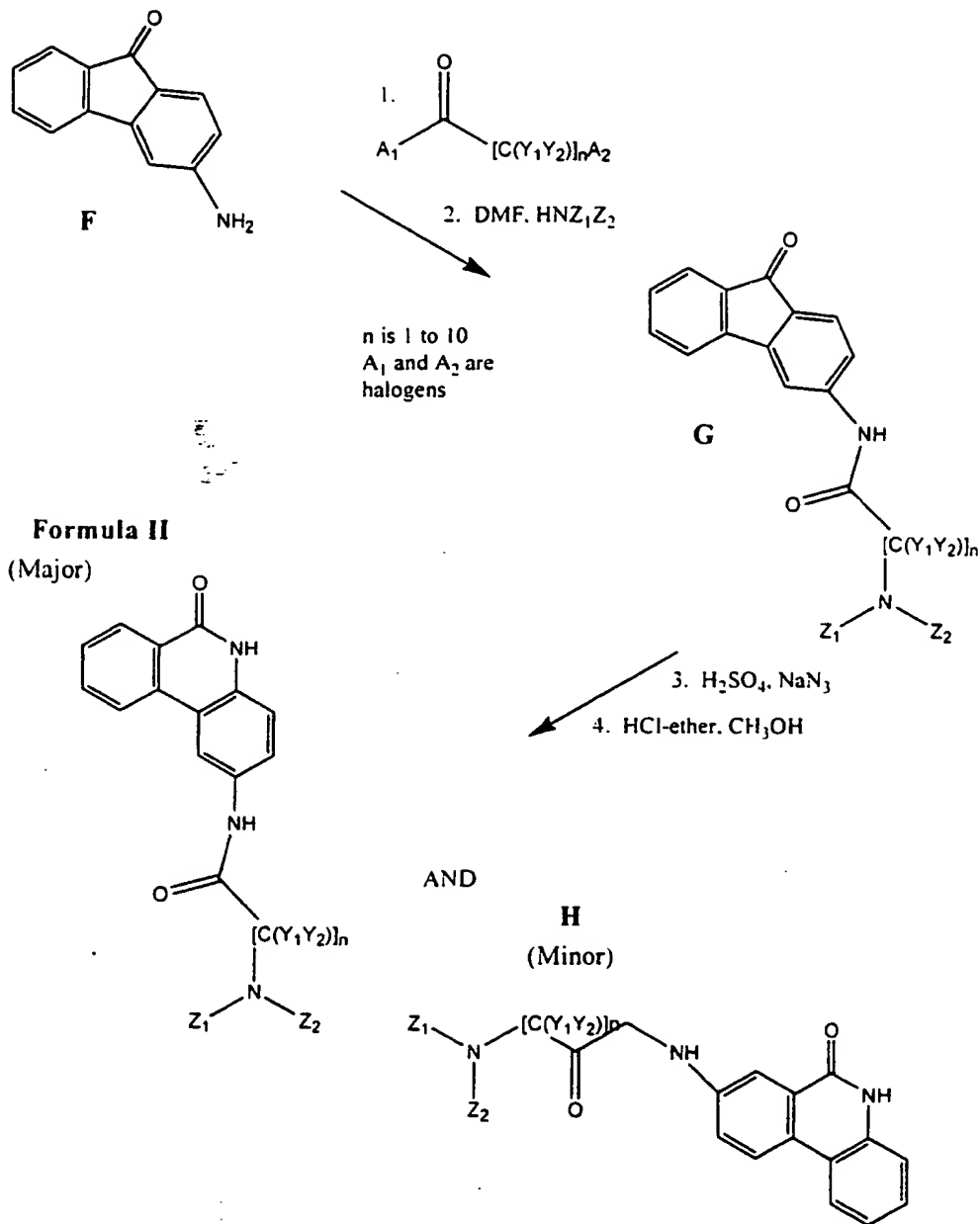
In Scheme I, a 9-fluorenone (A) is treated with sulfuric acid and aqueous sodium azide in a ring-expansion, or Schmidt, reaction to produce 6(5H)-phenanthridinone (B). Nitration of (B) produces 2-nitro-6(5H)-phenanthridinone (C) as a major product, which is then reduced by Fe, 10 NH_4Cl and DMF, or catalytic dehydrogenation, to produce 2-amino-6(5H)-phenanthridinone (D). In general, a compound falling within Formula II is prepared by modifying the parent molecule 6(5H)-Phenanthridinone. See, e.g., U.S. Patent Nos. 3,291,801 and 3,932,643; Taylor *et al.*, J. Am. Chem. Soc. 78:5104-5108, 1956. The 2-nitro (Compound C)-and 2-amino (Compound D)- 15 6(5H)-phenanthridinones are synthesized by a slight modification of procedures described in Andrievskii, *et al.*, Chem. Heterocycl. Compds (English Transl.) 21:8, 924-931, 1985, and Migachev, *et al.*, Chem. Heterocycl. Compds (English Transl.) 17:3, 394-397, 1981

Compound (E) is generated by acylating compound (D) with an appropriate halo-acylhalide. A compound falling within Formula II is then formed by the addition of a secondary 20 amine.

In Scheme II, 2-amino-9-fluorenone (compound (F)) is acylated with a halo-acylhalide to produce the N-acylated compound (G). The central ring of compound (G) is then expanded in a Schmidt reaction as described for Scheme I. The ring opening reaction produces a mixture of compounds: the 2-substituted compounds according to Formula II are the major component of 25 the mixture, while the 8-substituted compounds (H) are the minor component of the mixture.

Scheme I



Scheme II**EXAMPLES**

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims. The following examples illustrate the synthesis of novel substituted 6(5H)phenanthridinone derivatives of the invention and of their use to inhibit inflammation and reperfusion, and of the effects of substituted phenanthridinones on vascular

function *in vitro*, on *in vivo* in murine diabetes models, on organ transplantation and the effects on symptoms associated with neurodegenerative disorders.

Example 1: Synthesis of novel substituted phenanthridinones (Scheme I).

Synthesis of 6(5H)-phenanthridinone: 6(5H)-Phenanthridinone was prepared by the method described by Gauthier in U.S. Patent No. 3,932,643. Briefly, to a well stirred solution of 9-fluorenone (15 g, 0.083 mol) in concentrated sulfuric acid (500 mL), sodium azide (8.1 g, 0.12 mol) was slowly added over a period of 3 hr at 0 °C. The reaction mixture was stirred at room temperature for 2 hr, until nitrogen no longer evolved. The reaction mixture was then poured slowly over crushed ice, to produce a solid precipitate that was filtered and washed thoroughly with cold water to remove sulfuric acid. The solid was then dried under vacuum, to give pure 6(5H)-phenanthridinone (15g, 93%).

Synthesis of 2-Nitro-6(5H)-phenanthridinones 2-Nitro and 2-amino-6(5H)-phenanthridinones were synthesized by slight modification in the procedure described by Andrievskii *et al.*, Chem. Heterocycl. Compds (English Transl.) 21:8, 924-931, 1985 and by Migachev *et al.*, Chem. Heterocycl. Compds (English Transl.) 17:3, 394-397, 1981. Briefly, to a well stirred solution of 6(5H)-phenanthridinone (4.5 g, 0.023 mol) in acetic acid (200 mL) nitric acid (10 mL) was added, and the reaction mixture was stirred at 100 °C for 2 hr. The solid that separated out was filtered and washed thoroughly with acetic acid and then by cold water to remove acidic impurities. The solid was then dried under vacuum, then recrystallized from DMF to give pure 2-nitro-6(5H)-phenanthridinone (3.8 g, 70%). The filtrate was diluted with cold water, and a yellow colored solid precipitated that was filtered and washed with water. The yellow solid was then dried under vacuum, and identified to be pure 4-nitro-6(5H)-phenanthridinone (650 mg, 12%).

Synthesis of 2-Amino-6(5H)-phenanthridinones: To a suspension of 2-nitro-6(5H)-phenanthridinone (3.8 g, 0.016 mol) in DMF (200 mL) was added an ammonium chloride solution (3%, 200 mL), followed by the addition of iron powder (22 g). The reaction mixture was stirred at 100° C for 1 hr. The residue was removed by filtration, and the filtrate was made acidic by adding dilute HCl (25%, 20 mL). A solid separated from the solution and was filtered and washed thoroughly with cold water to remove acidic impurities. The solid was then dried under vacuum, to give hydrochloride salt of 2-amino-6(5H)-phenanthridinone (3.4 g, 89%).

Synthesis of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-chloroacetamide: To a suspension of HCl salt of 2-amino-6(5H)-phenanthridinone (3.4 g, 0.014 mol) in ethyl acetate (200 mL) was added saturated solution of sodium bicarbonate (200 mL), followed by addition of chloroacetyl chloride (5.6 mL, 0.07 mol). The reaction mixture was stirred at room temperature for 2 days. The solid that separated out was filtered and washed thoroughly with cold water. It was then dried under vacuum to give N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-chloroacetamide (3.35 gm, 85%).

According to the methods illustrated above, by changing the identity of the secondary amine, a number of different N,N-disubstituted compounds were produced. Representative compounds are shown in Table 1.

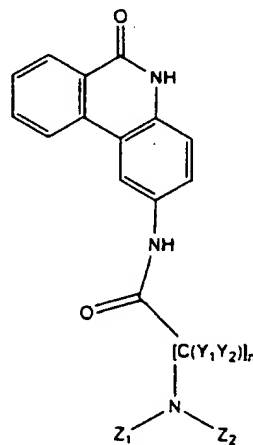
Specifically, PJ34 was prepared as follows:

A suspension of 2-nitro-6(5H)-phenanthridinone (C) in DMF was reduced by iron and ammonium chloride to produce 2-amino-6(5H)-phenanthridinone. Chloroacylation of 2-amino-6(5H)-phenanthridinone with chloroacetyl chloride in pyridine/DMF yielded N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-chloroacetamide, which then was treated with dimethyl amine in methanol or with N,N-dimethylformamide to furnish the N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide. This latter compound was then treated with HCl in ether in presence of methanol to give PJ34 as a water-soluble HCl salt. Product identity and purity were assessed using ¹HNMR, MS, TLC and HPLC.

20

TABLE I

Compound	n	Y ₁	Y ₂	Z ₁	Z ₂
PJ-34	1	H	H	-CH ₃	-CH ₃
PJ-44	1	H	H	-CH ₂ CH ₃	-CH ₂ CH ₃
PJ-36	1	H	H	-CH ₂ CH ₂ CH ₂ CH ₂ -	-
PJ-38	1	H	H	-CH ₂ CH ₂ OCH ₂ CH ₂ -	-
PJ-46	1	H	H	-CH ₂ CH ₂ N(CH ₃)CH ₂ CH ₂ -	-



Exmpl 2: Alternate synthesis of novel substituted phenanthridinones (Scheme II).

Synthesis of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-chloroacetamide: To a suspension of the HCl salt of 2-amino-6(5H)-phenanthridinone (100 mg, 0.04 mmol) in DMF (5 mL), pyridine (0.5 mL) was added, followed by the addition of chloroacetyl chloride (0.2 mL, 0.002 mol) at 0 °C. The reaction mixture was stirred at room temperature for 1 to 2 hrs. It was then poured over crushed ice, and the solid obtained was washed thoroughly with cold water. The solid was then dried under vacuum to give N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-chloroacetamide (95 mg, 84%).

Synthesis of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N, N-dimethylacetamide: To a solution of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-chloroacetamide (1.5 g, 0.0052 mol) in DMF (10 mL) a solution of dimethyl amine in methanol (20 mL) was added. The reaction mixture was stirred at room temperature for 3 hours. The solid that separated out was filtered and washed thoroughly with cold water. It was then dried under vacuum and recrystallized from methanol/ether to give N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide. This compound was dissolved in methanol (25 mL) and treated with a solution of HCl in ether (2 M, 5 mL). The solid was filtered and washed thoroughly with dry ether, and recrystallized from methanol/ether to provide the hydrochloride salt of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N, N-dimethylacetamide (1.4 g, 81%).

Synthesis of 2-(N-chloroacetyl)-9-fluorenone: A saturated solution of sodium bicarbonate (5 mL) was added to a solution of 2-amino-9-fluorenone (190 mg, 0.97 mmol) in ethyl acetate (5 mL). Next, chloroacetyl chloride (0.4 mL, 4.8 mmol) was added, and the reaction mixture was stirred at room temperature for 1 hour. The ethyl acetate layer was separated, and washed thoroughly with cold water. It was then dried under vacuum to give 2-(N-chloroacetyl)-9-fluorenone (185 mg, 70%).

Synthesis of 2-[N-(2-N,N-dimethylaminoacetyl)]-9-fluorenone: To a solution of 2-(N-chloroacetyl)-9-fluorenone (185 mg, 0.68 mmol) in DMF (5 mL) a solution of dimethyl amine in methanol (5 mL) was added. The reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was then poured over crushed ice and extracted in ethyl acetate (25 mL), washed thoroughly with cold water, dried over Na₂SO₄, then evaporated under vacuum to give 2-[N-(2-N,N-dimethylaminoacetyl)]-9-fluorenone (190 mg, 99%).

Synthesis of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamid : To a well stirred solution of 2-[N-(2-N,N-dimethylaminoacetyl)]-9-fluorenone (190 mg, 0.67 mmol) in concentrated sulfuric acid (5 mL), sodium azide (45 mg, 1.2 mmol) was added at 0° C. and the reaction mixture was stirred at room temperature until nitrogen no longer evolved. The reaction
5 was then poured slowly over an ice-cold K₂CO₃ solution, followed by extraction with ethyl acetate (25 mL). The ethyl acetate layer was washed thoroughly with cold water, dried over sodium sulfate, and concentrated under vacuum. The resulting residue was purified by PTLC (silica gel, 1000 µM) to give a mixture of N-(6-oxo-5,6-dihydro-phenanthridin-8-yl)-N,N-dimethylacetamide (52 mg, 26%) and N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-
10 dimethylacetamide (78 mg, 39%).

Example 3: Effects of substituted phenanthridinones on *in vitro* inflammation models

In *in vitro* studies, J774 macrophages were exposed to bacterial lipopolysaccharide (LPS), to induce pro-inflammatory mediator production and cytotoxicity. In this assay, the PJ compounds tested (PJ 34, 36, 38, 44 and 46) inhibited the production of the pro-inflammatory
15 cytokine tumor necrosis factor alpha (TNF-α), the production of the pro-inflammatory chemokines macrophage inhibitory factor-1α and -2 (MIP-1α and MIP-2), as well as the production of the pro-inflammatory free radical nitric oxide, measured here as nitrite. Furthermore, the compounds restored the viability of the cells, which was suppressed in response to LPS exposure. This assay (the LPS-stimulated macrophage) represents an *in vitro* model of an
20 inflammatory situation. Agents that block inflammatory mediator production in this assay are expected to have anti-inflammatory or immunosuppressive effects in inflammatory or immune diseases.

The half-maximal inhibitory effect (in µM) of various PJ compounds was assessed under a variety of conditions in immunostimulated J774 macrophages and in peroxynitrite-stimulated
25 thymocytes. Macrophages or thymocytes were first treated with a chosen PJ compound for 30 min at a concentration ranging from 0.1 to 30 µM. The cells were then immunostimulated with LPS (10 µg/ml) or treated with peroxynitrite (30 µM). MIP production in the macrophages was measured at 3h. TNF and nitric oxide were measured at 24h. cell viability in the macrophages were measured at 24h. PARS activation and cell death in thymocytes were measured at 6h.
30 Measurements of mediator production, PARS activation and cell death were essentially as described in Németh, *et al.*, Eur J Pharmacol 339:215-221, 1997 and Virág and Szabó, Br J Pharmacol, 126: 769-777, 1999. Results in Table 2 are shown as EC50 values (half maximal

inhibition of mediator production or restoration of cell viability: in μM): [mean \pm SEM of $n=3-6$ determinations].

TABLE 2

	Inhibition of TNF- α (J774)	Inhibition of MIP-1 α (J774)	Inhibition of MIP-2 (J774)	Inhibition of Nitrite (J774)	Enhancement of cell Viability (J774)	Inhibition of PARS activation (thymocyte)	Enhancement of cell viability (thymocyte)
PJ 34	5.4 ± 0.6	10.2 ± 1.4	7.2 ± 0.88	15.2 ± 2.8	12.5 ± 1.4	0.3 ± 0.1	0.2 ± 0.1
PJ 36	11.2 ± 1.1	15.2 ± 2.1	21.2 ± 2.7	25.4 ± 2.9	20.2 ± 2.3	0.2 ± 0.1	0.3 ± 0.1
PJ 38	6.2 ± 0.42	11.3 ± 1.4	14.9 ± 1.3	20.2 ± 1.5	22.1 ± 2.7	0.8 ± 0.2	0.4 ± 0.2
PJ 44	11.1 ± 0.67	10.2 ± 0.95	22.4 ± 1.2	No effect	16.2 ± 1.9	0.3 ± 0.1	0.2 ± 0.1
PJ46	9.2 ± 0.88	>30	>30	>30	>30	1 ± 0.3	1.3 ± 0.3

5 Example 4: Effects of substituted phenanthridinones on *in vitro* reperfusion injury models

In additional *in vitro* studies in isolated thymocytes, cells were exposed to peroxynitrite or hydrogen peroxide (as toxic oxidant species) to induce cytotoxicity. In this system the toxicity is, at least partially, related to activation of the nuclear enzyme PARS (see Introduction). In this assay, the compounds of the invention (*e.g.*, PJ 34, 36, 38, 44 and 46) inhibited the
 10 activation of PARS. Furthermore, the compounds prevented the oxidant-induced suppression of the viability of the cells (Table 2). The oxidant-stimulated thymocyte assay represents an *in vitro* model of a situation where cells are dying because of exposure to pro-oxidant species, as occurs in during the reperfusion of ischemic organs. A second experimental system was used where oxygen/glucose deprivation induced cell death in cultured neurons (*i.e.* an *in vitro* model of
 15 stroke). Here, PJ34 provided significant cytoprotection (up to 70%) in the concentration range of 10 nM-1 μM . Agents that block PARS activation or cell death in this assay are expected to have cytoprotective effects in various diseases associated with reperfusion of ischemic organs.

Example 5: Effect of substituted phenanthridinones on *in vivo* inflammation models

In order to substantiate the efficacy of the PJ series of compounds in inflammatory
 20 conditions, the effect of compounds according to the invention were tested in a systemic inflammatory model induced by bacterial lipopolysaccharide. Injection of bacterial lipopolysaccharide (LPS) at high doses causes multiple organ dysfunction resembling of septic

shock, and, ultimately, death. Agents that inhibit inflammatory mediator production, PARS activation, and cell death according to this model will prevent mortality induced by LPS. In experiments with Balb/c mice, injection of 90 mg/kg LPS intraperitoneally caused death in 92% of the animals over 24h. However, pretreatment of the animals with 20 mg/kg PJ34 reduced the
5 endotoxin-induced mortality to 50% under the same experimental conditions. In response to an even higher dose of LPS (120 mg/kg), the compounds of the invention (e.g., PJ 34, 36, 38, 44 and 46, each injected at 10 mg/kg every 6 hours) caused an improvement in the endotoxin-induced mortality from 70% death to 30-40% death over 24 hours.

These data indicate that the PJ series of compounds have therapeutic effects in various
10 systemic and local inflammatory conditions.

Example 6: Effect of substituted phenanthridinones on *in vivo* reperfusion injury models

In order to substantiate the efficacy of the PJ series of compounds in ischemia-reperfusion conditions, the effect of the compounds in a local model of reperfusion injury induced by ligation and release of the coronary artery in an anesthetized rat was examined. In a
15 model of 1-hour coronary ischemia, followed by reperfusion for 1 hour, treatment with PJ34 (5 g/kg intravenously (i.v.), injected 10 min prior to the start of reperfusion), reduced myocardial infarct size development from 62 ± 1 to 50 ± 3 % (area of necrosis, over area of risk). In addition, plasma levels of creatine phosphokinase (CPK, an indicator of myocardial necrosis) were reduced by PJ34 treatment by approx. 50%.

20 In another model, a mouse model of ischemic and reperfused gut was utilized. The superior mesenteric artery was occluded for 45 min, followed by a reperfusion for 1 h. At the end of the reperfusion, gut permeability was measured with the PD4 method in everted gut sacks. Ischemia-reperfusion increased the permeability of the gut from 9 ± 2 to 135 ± 27 ml/min/cm², indicating the severe damage of the reperfused gut. Treatment with PJ 34 (5 g/kg i.v., injected
25 10 mm prior to the start of reperfusion), reduced the increase in the permeability of the gut to 41 ± 12 ml/min/cm², indicating maintenance of the gut function. The ischemia-reperfusion studies in the gut were associated with a 38% mortality, whereas 100% survival was noted in the animals treated with PJ34. Similar protection was also observed with treatment with PJ36.

In another set of experiments, the effect of PJ34 in a rat model of middle cerebral artery
30 occlusion/reperfusion was tested. Occlusion lasted for 2 hours, followed by reperfusion for 24 hours. Infarct size was quantified with tetrazolium staining, and survival and neurological scores were monitored. PJ34 was administered at 10 mg/kg i.v. at 5 min before the start of reperfusion

in one group, and at 2h after the beginning of reperfusion in another group. Control or vehicle-treated animals developed a 73% mortality over 24 hours, whereas no animals died in the drug-treated groups. PJ34, given at the time of reperfusion or at 2h after the start of reperfusion reduced the size of brain infarction by 74 ± 6 and $55 \pm 8\%$, respectively. Vehicle treated animals
5 that survived for 24h developed severe neurological deficit (4 on a scale of 1-4), whereas no detectable deficit was observed in the animals treated with PJ34.

These data indicate that the PJ series of compounds have therapeutic effects in various systemic and local conditions of ischemia-reperfusion.

10 **Example 7: Effect of substituted phenanthridinone PJ34 *in vivo* on vascular function in a murine diabetes model**

In the studies described in Example 7 and Example 8, all materials were purchased from Sigma/Aldrich (St Louis, MO), unless specified otherwise.

Results are reported as mean \pm SEM of several experiments. Analysis of variance with Bonferroni's correction, or Student's *t*-test were used to compare mean values, as appropriate.
15 Statistical differences were declared significant for $p < 0.05$.

Male Balb/c mice were treated intraperitoneally (i.p.) with streptozotocin (240 mg/kg dissolved in citrate buffer) or vehicle (citrate buffer). Blood glucose was then monitored weekly over 8 weeks using a one-touch blood glucose meter (Lifescan). Hyperglycemia was defined as non-fasting blood glucose level higher than 200mg/dL.

20 Total glycated hemoglobin (GHb) content of blood samples was measured using a commercially available kit (Sigma Diagnostics) according to manufacturers instructions. Blood (50 μ l) was hemolyzed in 400 μ l of hemolyzing reagent and 50 μ l of the lysate was separated on an affinity resin column into a wash fraction (W) containing non-glycated hemoglobin and an elution fraction (E) containing glycated hemoglobin. The optical density of the two fractions
25 was measured by spectrophotometry at 415nm and the per cent glycated hemoglobin content was calculated as follows: $\text{GHb (\%)} = 100\text{E}/(\text{E} + 10\text{W})$.

Samples of pancreas were removed on week 8 and weighed before being placed into 6 ml of acid ethanol (23:7:0.45, ethanol: dH_2O :HCl) and homogenized. The pancreas was incubated for 72 h at 4 $^\circ\text{C}$ before being centrifuged. The insulin content of the supernatant was then
30 determined using a commercially available ELISA kit (Alpco).

After eight weeks, diabetic mice (treated with vehicle or with PARS inhibitor) were killed and thoracic aortae cleared from periadvential fat, cut into small pieces (typically 4 pieces) and placed into chambers filled with Krebs' solution [CaCl_2 1.6 (mM), MgSO_4 1.17 (mM), EDTA 0.026 (mM), NaCl 130 (mM), NaHCO_3 14.9 (mM), KCl 4.7 (mM), KH_2PO_4 1.18 (mM), glucose 11 (mM)], warmed to 37 °C, and gas equilibrated (95% O_2 , 5% CO_2). Isometric tension was measured with isometric transducers (Kent Scientific Corporation, Litchfield, CT), digitized using a MacLab A/D converter and stored and displayed on a MacIntosh computer. The pre-load was 1 g and the aortic rings were equilibrated for 60 minutes, the solution was changed every 15 minutes. Dose-response curves to the endothelium-dependent relaxant acetylcholine (10^{-9} to 3×10^{-4} M) were performed in rings precontracted with phenylephrine (10^{-6} M).

Complete destruction of pancreatic islet cells in mice was induced by treatment with high dose streptozotocin. This intervention induced a complete loss of pancreatic insulin content. Severe hyperglycemia ensued, which was persistent at plasma glucose levels of 250-350 mg/dl throughout the 8 weeks of the experiments (FIGS. 1A and 1B).

FIG. 1A shows blood glucose levels, blood glycosylated hemoglobin levels and pancreatic insulin content at 0-8 weeks after streptozotocin induced diabetes in male Swiss mice. Vehicle or PARS inhibitor treatment started at 1 week after streptozotocin (start of drug treatment indicated in the figure by an arrow), and continued throughout the experimental period. Drug treated animals were treated with 10 mg/kg PJ34 (*per os*), daily, once a day. At 8 weeks, blood was taken for measurement of glycosylated hemoglobin levels, pancreata taken for measurements of pancreatic insulin content, and thoracic aortae taken for determination of endothelium-dependent relaxant reactivity. There was no difference in the degree of hyperglycemia and glycosylated hemoglobin, and in the degree of pancreatic insulin loss between vehicle and PJ34 treated diabetic animals.

FIG. 1B demonstrates acetylcholine induced, endothelium-dependent relaxations in epinephrine-precontracted isolated thoracic aortic rings from animals which received no streptozotocin injection ("Control"), diabetic animals at 8 weeks treated with vehicle ("8 week - Vehicle"), and diabetic animals treated with PJ34 ("8 week- PARS inhibition"). There was a marked impairment of the endothelium-dependent relaxant ability of the vascular rings in diabetes, which was normalized in the animals where PARS was inhibited. Chronic treatment of normal (non-diabetic) mice with the PARS inhibitor (regimen as above) for 8 weeks did not affect the vascular reactivity (not shown). ** $P < 0.01$ for vehicle-treated vs. PJ34 treated diabetic mice. N=8 animals or rings per group.

In a second group of mice, inhibition of PARS activation was achieved by chronic treatment with PJ34, starting at 1 week after the initiation of diabetes. After 8 weeks of hyperglycemia, there was an increase in immunostaining for nitrotyrosine (a reactive species produced from the reaction of superoxide and nitric oxide, in the vascular rings, and massive endothelial staining for poly(ADP-ribose), indicative of PARS activation. Ischiropoulos *et al.*, Arch. Biochem. Biophys. 298: 431-7, 1992; Halliwell, FEBS Lett. 411: 157-60, 1997. Furthermore, *ex vivo* experiments demonstrated the loss of endothelial function, as measured by the relaxant responsiveness of pre-contracted vascular rings to the endothelium dependent vasodilator, nitric oxide liberating hormone acetylcholine (FIGS. 1A and 1B). Furchgott, Acta
10 Physiol. Scand. 139: 257-70, 1990.

Immunohistochemical staining for nitrotyrosine, an indicator of the generation of reactive nitrogen species, was also examined in control rings, in rings from diabetic animals treated with vehicle at 8 weeks, and in rings from diabetic animals treated with PJ34. There was a marked increase in tyrosine nitration in the diabetic rings, which was not affected by PJ34 treatment.

15 Immunohistochemical staining was also performed to look at levels of poly(ADP-ribose), as an indication of PARS activation. Staining was examined in control rings, in rings from diabetic animals treated with vehicle at 8 weeks, and in rings from diabetic animals treated with PJ34. There was a marked increase in PARS activation in diabetic endothelium, which was abolished by PJ34 treatment. Similar immunohistochemical profiles were seen in n=4-5 vascular
20 rings in each experimental group.

Delayed treatment with a PARS inhibitor ameliorated poly(ADP-ribose) staining and restored normal vascular function in diabetic mice, without altering systemic glucose levels, plasma glycated hemoglobin levels, or pancreatic insulin content. Respective EC_{50} values for acetylcholine amounted to $0.11 \pm 0.08 \mu M$, $0.83 \pm 0.76 \mu M$ and $0.55 \pm 0.49 \mu M$ in control, diabetic
25 and PJ34 treated diabetic rings, respectively. Thus, the EC_{50} value of acetylcholine was not altered by PJ34, indicating that its protective effect is independent of altering the endothelial receptor sensitivity to acetylcholine.

Example 8: Effect of the substituted phenanthridinone PJ34 *in vitro* in cells from PARS^{+/+} and PARS^{-/-} mice

Human umbilical vein endothelial cells (HUVECs), and pulmonary endothelial cells generated from wild type and PARS^{-/-} mice were placed in high glucose medium or an
5 osmotically controlled normal glucose control.

Breeding pairs of mice for the PARS colony were a gift from Dr. Z.Q. Wang, International Agency for Research on Cancer Lyon, France. Thoracic aortae from PARS^{-/-} and PARS^{+/+} mice (Wang *et al.*, Genes Dev.9: 509-520, 1995) were harvested, cleared of periadvential fat, and cut into 3 or 4 pieces. Rings were placed in tissue culture plates containing
10 different media preparations (as below) and the plate was incubated at 37 °C and 5% CO₂ for 24 hours. Four different media preparations were used: a) normal glucose, *i.e.* 5 mM; in F-12K Nutrient Mixture medium (Gibco BRL – Life Technologies); b) Osmotic control, *i.e.* 30 mM L-glucose in F-12K medium; c) High glucose *i.e.* 30 mM D-glucose in F-12 K medium; and d) PARS inhibitor in the presence of high glucose: 3 µM PJ34 and 30 mM D-glucose in F-12K
15 medium.

After incubation, aortic rings were mounted in organ baths filled with warmed and gas equilibrated Krebs' solution and endothelium dependent relaxant ability was evaluated as described above. Osmotic controls (30 mM L-glucose) were indistinguishable from normal glucose controls and are not presented in the figures.

20 To detect PARS, paraffin sections (3 µm) were deparaffinized in xylene and rehydrated in decreasing concentrations (100%, 95% and 75%) of ethanol followed by a 10 min incubation in PBS (pH 7.4). Sections were treated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity and then rinsed briefly in PBS. Non-specific binding was blocked by incubating the slides for 1h in PBS containing 2% horse serum. To detect poly(ADP-
25 ribose), a routine histochemical procedure was applied, as previously described (Scott *et al.*, Ann. Neurol. 45: 120-124, 1999) with minor modifications, as follows: Mouse monoclonal anti-poly(ADP-ribose) antibody (Alexis, San Diego, CA) and isotype-matched control antibody were applied in a dilution of 1:100 for 2h at room temperature. Following extensive washing (5 X 5 min) with PBS, immunoreactivity was detected with a biotinylated goat anti-rabbit secondary
30 antibody and the avidin-biotin-peroxidase complex (ABC), both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, CA). Color was developed using Ni-DAB substrate (95 mg diaminobenzidine, 1.6 g NaCl, 2 g nickel sulfate in 200 ml 0.1M acetate buffer). Sections were

then counterstained with nuclear fast red, dehydrated and mounted in Permount.

Photomicrographs were taken with a Zeiss Axiolab microscope equipped with a Fuji HC-300C digital camera.

Detection of nitrotyrosine in aorta rings was carried out as described above for
5 poly(ADP-ribose) staining except that rabbit anti-nitrotyrosine polyclonal antibody was used as the primary antibody. For nitrotyrosine staining of endothelial cells, the cells were fixed in ice cold 10% trichloroacetic acid (10 min) followed by incubations in 70%, 95%, and 100% ethanol for 3min each. Cells were rehydrated in PBS, blocked in 2% goat serum (1h, room temperature) and incubated in anti-nitrotyrosine (1:100dilution) for 2h. Subsequent steps of the staining
10 procedure were identical to the tissue staining. Color was developed with Vector Red substrate, and slides were mounted in glycerol.

In vitro studies in endothelial cells were performed by preparing cell as described in Gerritsen *et al.*, Microcirculation 2:151-163, 1995. In brief, lung microvascular endothelial cells were isolated from wild-type (+/+) and PARS knockout (-/-) mice. Cells were maintained in
15 F12/DMEM (1:1) medium supplemented with endothelial cell growth supplement (100 µg/l heparin and 10% fetal calf serum).

Human umbilical vein endothelial cells (HUVEC) were obtained from ATCC and grown in F12 medium supplemented with endothelial cell growth supplement, 100 µg/l heparin and 10% fetal calf serum.

20 PARS activation was measured in endothelial cells seeded in 6 well tissue culture plates pre-coated with endothelial cell attachment factor (Sigma). Medium was replaced by regular- (see above) or FCS-free HUVEC medium containing 5 mM D-glucose 12h before the experiment. Cells were pretreated with MnTBAP (100 µM), carboxy-PTIO (300 µM), L-NMMA (3 mM), L-NAME (3 mM) or PJ34 (0.1 - 3 µM) for 30 min followed by 6h incubation
25 in 30 mM glucose. At the end of incubation, medium was removed and PARS buffer [56 mM Hepes pH 7.5, 28 mM KCl, 28 mM NaCl, 2 mM MgCl₂, 0.01 % digitonin and 0.125 µM ³H-NAD (0.5 µCi/ml)] was added to the cells. After 10 min, cells were scraped, transferred into Eppendorf tubes and the reaction was stopped by trichloroacetic acid (TCA). Pellets were washed three times in 5% TCA and then solubilized in 500 µl 2% SDS/0.1 N NaOH at 37 °C.
30 Contents of the tubes were added to 7 ml ScintiSafe Plus scintillation liquid and radioactivity was measured in a liquid scintillation counter (Wallac, Gaithersburg, MD). Activity was normalized for protein content.

High glucose concentration over 2-6 hours induced the activation of PARS in the murine endothelial cells, an effect not observed in the PARS^{-/-} endothelial cells or in HUVECs treated with PJ34. The results are shown in FIGS. 2A-2E.

FIG. 2A shows a time course of PARP activation and cell viability in wild-type and
5 PARP deficient endothelial cells exposed to high glucose (30 mM), when compared to activity in normal culture medium (5 mM glucose). There was a significant, prolonged activation of PARP in response to high glucose. PARP^{-/-} cells showed no detectable increase in PARP activity. The osmotic control (30 mM L-glucose) did not alter PARP activity. The right side of the figure shows the inhibitory effect of the cell-permeable superoxide dismutase mimic MnTBAP (100
10 μ M), and the nitric oxide scavenger carboxy-PTIO (300 μ M) and the inhibitory effect of the PARP inhibitor PJ34 (100 nM - 3 μ M) on high glucose induced PARP activation, at 6 hours.

FIG. 2B shows activation of PARP in response to high glucose (30 mM), when compared to activity in normal culture medium (5 mM) in human umbilical vein endothelial cells (HUVECs) at 24 hours, and inhibitory effect of PJ34 (3 μ M). The osmotic control (30 mM L-
15 glucose) did not alter PARP activity or cell viability (not shown).

Tyrosine nitration was observed in endothelial cells maintained in normal culture medium (5 mM glucose) and in cells exposed to high glucose (30 mM). There was an intense nitrotyrosine staining in endothelial cells in high glucose, with a preferential perinuclear localization.

20 The development of endothelial dysfunction in thoracic aortic rings obtained from wild-type mice is illustrated in FIG. 2D. Rings incubated in 30 mM glucose for 16 hours showed an impairment in endothelium-dependent relaxation; an effect not seen in rings from PARP^{-/-} mice, and an effect prevented by co-incubation of the wild-type rings with PJ34 (1 μ M) in the presence of high glucose ("*High glucose + PARP inhibition*"). The osmotic control (30 mM L-glucose)
25 did not alter endothelium-dependent relaxations (not shown). n=5-6 independent experiments. *p<0.05 between groups as indicated.

FIG. 2C shows a proposed scheme for the mechanism of PARP activation in diabetic blood vessels. Consistent with this scheme, high glucose induced the activation of PARS in human umbilical vein endothelial cells. PARS activation induced by high glucose concentration
30 was prevented by PJ34, and was markedly reduced in mice of the PARS negative phenotype (FIGS. 2A and 2B). A small degree of increase in PARS activation (36% above control at 6h of

glucose incubation) was nevertheless also observed in the PARS^{-/-} endothelial cells, which may be related to the existence of the various minor isoforms of PARS (see below).

Similar to the *in vivo* situation in diabetes mellitus, tyrosine nitration was detected in the endothelial cells exposed to high glucose. PARS activation in response to high glucose was
5 attenuated by MnTBAP, a cell-permeable water-soluble superoxide dismutase mimic, as well as by carboxy-PTIO, a nitric oxide scavenger compound, indicating that the activation of PARS is related to intravascular generation of reactive oxygen and nitrogen species (FIG. 2A). Similarly, pharmacological inhibition of cellular nitric oxide synthesis by N^G-methyl-L-arginine (L-NMA, 3 mM) or N^G-nitro-L-arginine methyl ester (L-NAME, 3 mM) significantly reduced the degree of
10 PARS activation in endothelial cells exposed to high glucose.

Similar to the *in vivo* situation in hyperglycemia and diabetes, incubation of thoracic aortae from wild-type mice in high glucose concentration for 16 hours resulted in a significant loss of contractile and endothelium-dependent function. Vascular rings from the PARS^{-/-} mice, in contrast, were fully resistant against high glucose induced endothelial dysfunction (FIG. 2C).
15 Similarly, pharmacological inhibition of PARS also protected against the development of high glucose induced endothelial dysfunction (FIG. 2C). As in the *ex vivo* studies (see above), acetylcholine sensitivity of the rings incubated in high glucose was not altered by PARS inhibition or deficiency (not shown).

Example 9: Effect of the substituted phenanthridinone PJ34 on transplanted heart 20 function in an animal model system

The effect of PARS inhibition on basal and acetylcholine-stimulated coronary blood flow in a rat heart transplantation model was examined using PJ34.

In this model, there is a transient suppression of myocardial blood flow and mechanical performance of the transplanted heart, which occurs as a result of external storage and
25 subsequent reperfusion of the transplanted heart. Cardiac function is generally restored within 24 hours post-transplantation. Agents that are beneficial in this model accelerate recovery of the endothelial and cardiac function.

Studies were performed generally as described in Szabo *et al.*, Cardiovasc. Res. 39: 683-90, 1998. Briefly, isogenic intra-abdominal heterotopic transplantation was performed in Lewis
30 rats. After 1 hour of cold ischemic preservation, heart transplantation was performed. Circulation was restored either after application of placebo (control), or the PARS inhibitor PJ34

(3 mg/kg). An implanted balloon was used to obtain pressure-volume relations of the transplanted heart. Myocardial blood flow (MBF) was assessed by the hydrogen-clearance method. Measurements were taken after 1 and 24 h after transplantation. Endothelium-dependent vasodilation in response to acetylcholine (ACH) and endothelium-independent vasodilation in response to sodium nitroprusside were also determined.

Acetylcholine-induced vasodilation in transplanted or PJ34-treated hearts is shown in Tables 3 and 4. Effect of administration of the PARS inhibitor PJ34 (3 mg/kg i.v., immediately after the heart transplantation), on blood flow (basal and acetylcholine stimulated) in heterotopic rat hearts, at 1h and 24h after transplantation is shown. Inhibition of PARS with PJ34 markedly improved basal and acetylcholine stimulated coronary blood flow at 1h after transplantation in this model. Administration of the PARS inhibitor PJ34 also markedly enhanced the myocardial performance (data not shown). The function of the hearts after 1h was comparable to (*e.g.*, not significantly different from) the function of the vehicle-treated hearts at 24 hours. These results demonstrate that inhibition of PARS brought about a near-complete functional recovery within 1 hour in this model of isogeneic heart transplant. These data indicate that inhibition of PARS by the compounds of the invention resulted in marked improvement of the function of the transplanted heart. Data shown represent N=5-6 per experimental group.

TABLE 3: Basal coronary blood flow (ml/min)

Time	Vehicle treated	PJ34 treated
1h after transplant	2.8±0.3	4.1±0.4
24h after transplant	3.9±0.3	4.2±0.2

TABLE 4: Acetylcholine stimulated coronary blood flow (ml/min)

Time	Vehicle treated	PJ34 treated
1h after transplant	3.5±0.3	6.5±0.5
24h after transplant	6.1±0.4	8.2±0.8

Example 10: Effect of the substituted phenanthridinone PJ34 on symptoms associated with experimental allergic encephalomyelitis in an animal model system

The effect of the substituted phenanthridinone PJ34 on alleviating symptoms associated with experimental allergic encephalomyelitis (EAE) in an animal model system was examined.

5 Studies of animals with experimental allergic encephalomyelitis (EAE) are generally considered to be the only available animal models of MS, sharing some clinical and histopathological features with the human disease. In these models, EAE is caused by the induction of an immune response to autoantigens from the myelin sheath of nerves, such as myelin basic protein (MBP). Symptoms can include temporary paralysis, or even death of the animals. The EAE models are

10 frequently used to study the pathogenesis of MS and to test novel therapeutic strategies aimed at treating MS.

The studies were performed according to standard methods. *See, e.g., Hooper et al., Proc Natl Acad Sci U S A 95:675-680, 1998.* Briefly, EAE was caused by immunization of 8-10 week old female SJL/PLJ mice with MBP (in complete Freund's adjuvant containing 0.05% M

15 Butyricum plus an additional 4mg/ml Mycobacterium Tuberculosis H37 RA, one 200 ml subcutaneous dose plus 2 doses of 400 ng pertussis toxin, intra-peritoneal, on days 0 and 2). The grading system shown in Table 5 was used to evaluate the severity of the disease.

Ten days after the start of the immunization, mice began to receive vehicle or PJ34 treatment (the latter at 10 mg/kg orally, 2 times daily). The results are shown in Tables 6 and 7.

20 PJ34 treated animals showed a reduction in the severity of the disease (Table 6), and reduction in mortality (Table 7). There was a significant reduction in severity and mortality in the PJ34 treated animals. These data demonstrate that pharmacological inhibition of PARS provides therapeutic benefit in EAE, MS and related chronic neurodegenerative diseases.

TABLE 5. KEY: Grading System For Clinical Assessment Of EAE:

Number	Global Characteristics	Descriptive Characteristics
0	Normal Mouse	
1	Piloerection, tail weakness	rough fur limited ability to curl tail unable to swing body from tail
2	Tail paralysis	tail hangs limply no problem with mobility can still support itself on cage bars or edge of cage with hind feet
3	Tail paralysis and hind limb weakness (forelimb weakness without hind limb or tail effect)	tail hangs limply waddling gait falls through cage bars with hind feet cannot walk along edge of cage
4	Tail and partial hind limb paralysis (forelimb partial paralysis without hind limb or tail effect)	tail hangs limply unable to maintain rump posture or walk on all fours falls through cage bars with hind feet cannot hang from edge of cage with hind feet some significant hind limb movement still apparent
5	Tail and complete hind limb paralysis (forelimb paralysis without hind limb or tail effect)	tail hangs limply no significant hind limb movement drags itself around on forelimbs food and water must be supplied on cage floor (long sipper tubes)
6	General Paralysis	no significant hind limb movement incapable of pulling itself around on forelimbs animal still alert some head movement with or without some forelimb movement food and water must be supplied on cage floor (long sipper tubes)
7	Moribund	Animal no longer alert Sacrifice for humane reasons = death from EAE

TABLE 6. Average Disease severity index (Days after immunization) in control and PJ34 treated mice subjected to EAE. ($p < 0.05$).

Day	10	11	12	13	14	15	16	17	18	19	20
Control	0	4	10	13	17	9	18	15	22	23	16
PJ34	0	0	0	0	0	0	4	4	4	4	3

5

TABLE 7. Percent mortality (Days after immunization) in control and PJ34 treated mice subjected to EAE. ($p < 0.05$).

Day	10	11	12	13	14	15	16	17	18	19	20
Control	0	0	0	0	20	20	30	30	30	40	40
PJ34	0	0	0	0	0	0	0	0	0	0	0

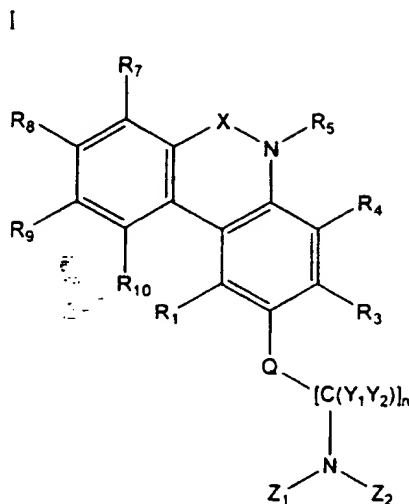
OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims.

5

What is claimed is:

1. A compound of formula I:



or a pharmaceutically acceptable base or acid addition salt, hydrate, ester, solvate, prodrug, metabolite, stereoisomer, or mixtures thereof, wherein:

X is C=O, C=S, SO₂, C=NH, C=NR₆, C-Cl;

Q is NHCO, O, CO, OCO₂, OCO, OCONH, NR₂, NHCO₂, S, SO₂, CS, SO;

R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, are, independently, hydrogen or lower alkyl;

Y₁ and Y₂ are, independently: hydrogen, halo, alkylhalo, hydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₁-C₈ carbocyclic, aryl, alkylamino, amino, carboxy, ester, arylalkyl, nitro;

n is 0 to 10; and

Z₁ and Z₂ are, independently: hydrogen, alkylhalo, alkylhydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₂-C₁₀ straight or branched chain alkynyl group, aryl, benzyl, alkylamino, alkylcarboxy, alkylester, arylalkyl, or Z₁ or Z₂ taken together form a fused ring, wherein said ring has 4-8 ring members.

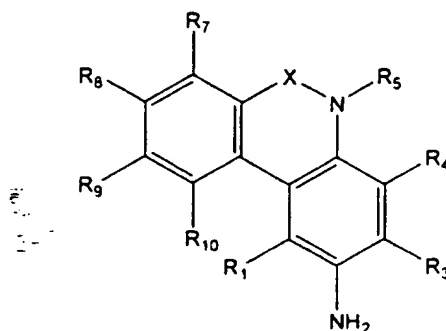
2. The compound of claim 1, wherein X is C=O.
3. The compound of claim 2, wherein R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, Y₁, and Y₂ are hydrogen.
4. The compound of claim 2, wherein Q is NHCO.
5. The compound of claim 4, wherein n is 1.

6. The compound of claim 5, wherein Z_1 and Z_2 are methyl groups.
7. The compound of claim 5, wherein Z_1 and Z_2 are ethyl groups.
8. The compound of claim 5, wherein Z_1 and Z_2 are isopropyl groups.
9. The compound of claim 5, wherein Z_1 is a methyl group and Z_2 is a benzyl group.
10. The compound of claim 5, wherein N , Z_1 and Z_2 taken together, form piperidine, piperazine, N-alkylated or alkylcarbonylated piperazine, pyrrole, imidazole, indole, or other C_3 to C_{10} branched or cyclic or cycloalkenyl amines.
11. The compound of claim 5, wherein Z_1 , N , and Z_2 taken together form a fused ring having six ring members.
12. The compound of claim 11, wherein Z_1 and Z_2 taken together form $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$.
13. The compound of claim 11, wherein one of the ring members is oxygen.
14. The compound of claim 13, wherein Z_1 and Z_2 taken together form $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_2-$.
15. The compound of claim 11, wherein two of the ring members are nitrogen.
16. The compound of claim 15, wherein Z_1 and Z_2 taken together form $-\text{CH}_2-\text{CH}_2-\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-$.

17. A method of making a modified phenanthridinone, the method comprising:

(a) providing a salt of a 2-amino-6(5H)-phenanthridinone having the structure of compound III:

III



wherein:

X is C=O, C=S, SO₂, C=NH, C=NR₆, C-Cl;

R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀ are, independently: Q-(CR₁₁R₁₂)_n-(NR₁₃R₁₄)_n;

Q is O, CO, OCO₂, OCO, OCONH, NH, NHCO, NHCO₂, S, SO₂, CS, or SO;

R₁₁ and R₁₂ are, independently: hydrogen, halo, alkylhalo, hydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₃-C₈ carbocyclic, aryl, alkylamino, amino, carboxy, ester, arylalkyl, nitro;

n is 0 to 10;

R₁₃ and R₁₄ are, independently: hydrogen, alkylhalo, alkylhydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₂-C₁₀ straight or branched chain alkynyl group, C₃-C₈ carbocyclic, aryl, benzyl, alkylamino, alkylamino, alkylcarboxy, alkylester, arylalkyl, or R₁₃ or R₁₄ are cyclic piperidine, piperazine, N-alkylated or alkylcarbonylated piperazines, pyrrole, imidazole, indole, and other C₂ to C₁₀ branched or cyclic or cycloalkenyl amines;

(b) acetylating said 2-amino-6(5H)-phenanthridinone with a haloacetyl halide under conditions which allow for the formation of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-haloacetamide; and

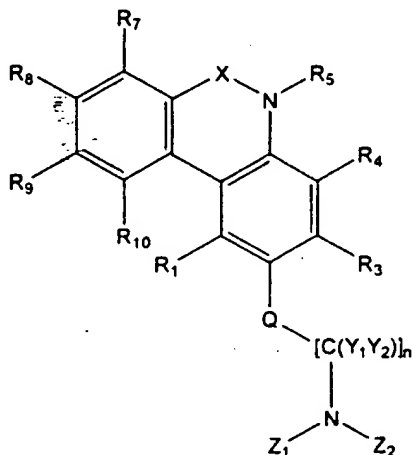
(c) reacting said N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-haloacetamide with HNZ₁Z₂ under conditions which allow for the formation of N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-Z₁Z₂acetamide, thereby forming a modified phenanthridinone.

18. The method of claim 17, wherein Z₁ and Z₂ are, independently, alkoxy, hydrogen, halo, alkylhalo, hydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched

chain alkenyl group, aryl, alkylamino, amino, carboxy, ester, arylalkyl, nitro, or Z_1 , N , and Z_2 taken together form a fused ring, wherein said ring has 4-8 ring members.

19. A method of inhibiting poly(ADP)-ribose synthase activity in a cell, the method comprising contacting said cell with a compound of Formula I in an amount sufficient to inhibit poly (ADP)-ribose-synthase in said cell:

I



or a pharmaceutically acceptable base or acid addition salt, hydrate, ester, solvate, prodrug, metabolite, stereoisomer, or mixtures thereof, wherein:

X is C=O, C=S, SO_2 , C=NH, C=NR₆, C-Cl;

Q is NHCO, O, CO, OCO_2 , OCO, OCONH, NR₂, $NHCO_2$, S, SO_2 , CS, SO;

R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, are, independently, hydrogen or lower alkyl;

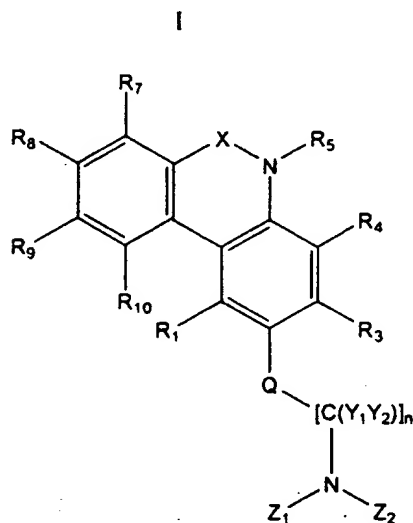
Y₁ and Y₂ are, independently: hydrogen, halo, alkylhalo, hydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₃-C₈ carbocyclic, aryl, alkylamino, amino, carboxy, ester, arylalkyl, nitro;

n is 0 to 10; and

Z₁ and Z₂ are, independently: hydrogen, alkylhalo, alkylhydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₂-C₁₀ straight or branched chain alkynyl group, aryl, benzyl, alkylamino, alkylcarboxy, alkylester, arylalkyl, or Z₁ or Z₂ taken together form a fused ring, wherein said ring has 4-8 ring members.

20. A method of treating or preventing local or systemic inflammation in a subject, the method comprising administering the compound of claim 1 in an amount sufficient to inhibit inflammation in said subject.

21. A method for treating or preventing a disease or condition relating to cardiovascular complications of diabetes in a subject, the method comprising administering to a subject in need thereof an amount of an inhibitor of a poly (ADP-Ribose) polymerase (PARS) sufficient to inhibit activation of said PARS.
22. A method for enhancing the function of a transplanted organ in a subject, the method comprising administering to said subject a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount sufficient to enhance the function of the transplanted organ in said subject.
23. A method for treating symptoms associated with multiple sclerosis in a subject in a subject, the method comprising administering to said subject a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount to alleviate symptoms of multiple sclerosis in said subject.
24. The method of any of claim 21, 22, or 23, wherein said PARS inhibitor is a compound of Formula I:



or a pharmaceutically acceptable base or acid addition salt, hydrate, ester, solvate, prodrug, metabolite, stereoisomer, or mixtures thereof, wherein:

X is C=O, C=S, SO₂, C=NH, C=NR₆; C-Cl;

Q is NHCO, O, CO, OCO₂, OCO, OCONH, NR₂, NHCO₂, S, SO₂, CS, SO;

R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, are, independently, hydrogen or lower alkyl;

Y₁ and Y₂ are, independently: hydrogen, halo, alkylhalo, hydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₃-C₈ carbocyclic, aryl, alkylamino, amino, carboxy, ester, arylalkyl, nitro;

n is 0 to 10; and

Z₁ and Z₂ are, independently: hydrogen, alkylhalo, alkylhydroxy, C₁-C₁₀ straight or branched chain alkyl, C₂-C₁₀ straight or branched chain alkenyl group, C₂-C₁₀ straight or branched chain alkynyl group, aryl, benzyl, alkylamino, alkylcarboxy, alkylester, arylalkyl, or Z₁ or Z₂ taken together form a fused ring, wherein said ring has 4-8 ring members.

25. The method of claim 24, wherein X is C=O.
26. The method of claim 25, wherein R₁, R₃, R₄, R₅, R₆, R₇, R₈, R₉, Y₁, and Y₂ are hydrogen.
27. The method of claim 25, wherein Q is NHCO.
28. The method of claim 27, wherein n is 1.
29. The method of claim 28, wherein Z₁ and Z₂ are methyl groups.
30. The method of claim 28, wherein Z₁ and Z₂ are ethyl groups.
31. The method of claim 28, wherein Z₁ and Z₂ are isopropyl groups.
32. The method of claim 28, wherein Z₁ is a methyl group and Z₂ is a benzyl group.
33. The method of claim 28, wherein N, Z₁ and Z₂ taken together, form piperidine, piperazine, N-alkylated or alkylcarbonylated piperazine, pyrrole, imidazole, indole, or other C₂ to C₁₀ branched or cyclic or cycloalkenyl amines.
34. The method of claim 33, wherein Z₁, N, and Z₂ taken together form a fused ring having six ring members.
35. The method of claim 34, wherein Z₁ and Z₂ taken together form -CH₂-CH₂-CH₂-CH₂-CH₂-.
36. The method of claim 34, wherein one of the ring members is oxygen.
37. The method of claim 36, wherein Z₁ and Z₂ taken together form -CH₂-CH₂-O-CH₂-CH₂-.
38. The method of claim 34, wherein two of the ring members are nitrogen.
39. The method of claim 38, wherein Z₁ and Z₂ taken together form -CH₂-CH₂-N(CH₃)-CH₂-CH₂-.

40. The method of claim any of claim 21, 22, or 23, wherein said inhibitor is selected from the group consisting of benzamide, nicotinamide, 3-aminobenzamide, 4-aminobenzamide, and 1,5, dihydroxy-isoquinoline.
41. The method of any of claim 19, 20, 21, 22, 23, or 40, wherein said administering is intravenous, intraperitoneal, intramuscular, intraventricular, subcutaneous, topical, sublingual, or oral.
42. The method of any of claim 20, 21, 22, or 23, wherein said subject is a human subject.
43. The method of claim 20 where said local inflammatory condition is caused by an inflammatory disorder of a joint, an inflammatory bowel disease, an inflammatory lung disorder, an inflammatory disease of the central nervous system, or an inflammatory disease of the eye.
44. The method of claim 20 where said systemic inflammatory condition is caused by a condition selected from the group consisting of gram-positive shock, gram negative shock, hemorrhagic shock, anaphylactic shock, traumatic shock, and systemic inflammation and chemotherapeutic shock.
45. The method of claim 21, wherein administering said inhibitor does not increase insulin levels in said subject.
46. The method of claim 21, wherein said diabetes is Type I diabetes.
47. The method of claim 21, wherein said diabetes is Type II diabetes.
48. The method of claim 21, wherein the disease or condition is selected from the group consisting of retinopathy, nephropathy, peripheral vascular disease, vascular aneurysm, limb ischemia, ischemia-related limb gangrene, ulcer, skin lesion, claudication, autonomic nervous system dysfunction, peripheral neuropathy, organic impotence, accelerated atherosclerosis, cardiomyopathy, myocardial dysfunction, ischemia, infarction, and cerebrovascular disease.
49. The method of claim 21, wherein the disease is peripheral vascular disease.
50. The method of claim 21, wherein the disease or condition is a foot ulcer.

51. The method of claim 22, wherein the transplanted organ is contacted with the inhibitor prior to introduction of the organ into said subject.
52. The method of claim 22, wherein the inhibitor is administered to said subject in conjunction with the introduction of the organ into said subject.
53. The method of claim 22, wherein the transplanted organ is selected from the group consisting of lung, liver, retina, pancreatic islet, blood vessel, skin, and bone.
54. The method of claim 22, wherein the transplanted organ is a kidney.
55. The method of claim 22, wherein the transplanted organ is a heart.
56. The method of claim 55, wherein the subject is a human.
57. The method of claim 22, wherein the inhibitor is applied in combination with one or more immuno-suppressants.
58. The method of claim 57, wherein at least one of said immuno-suppressants is selected from the group consisting of cyclosporine, tacrolimus, mycophenolate mofetil, corticosteroids, and azathioprine.
59. The method of claim 22, wherein the inhibitor is applied in combination with at least one organ preservative solution.
60. The method of claim 59, wherein said one or more organ preservative solution is selected from the group consisting of a histidine-tryptophan-alpha-ketoglutarate solution, Belzer's solution, Collins solution, EP4, St. Thomas solution and a Stanford solution.
61. The method of claim 23, further comprising administering one or more additional agents for treating symptoms associated with multiple sclerosis.
62. The method of claim 61, wherein the second agent is selected from the group consisting of a glucocorticoid, an interferon, glatiramer acetate, immunoglobulin, and mitoxantrone.
63. The method of claim 62, wherein the immunoglobulin is intravenous immune globulin.
64. A method of treating or preventing reperfusion injury in a subject, the method comprising administering the compound of claim 1 in an amount sufficient to inhibit reperfusion injury in said subject.

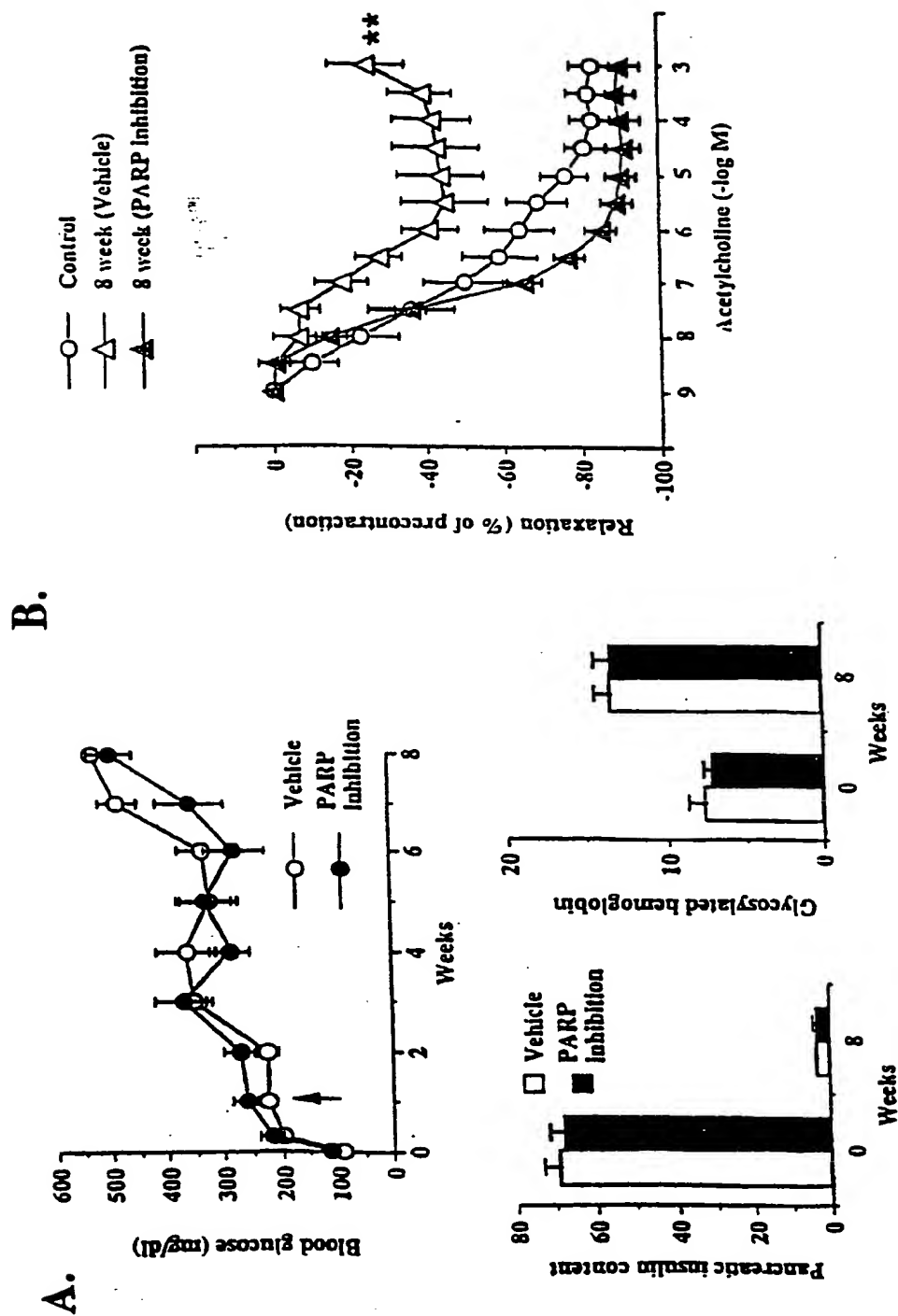
65. The method of claim 64, wherein said compound is administered prophylactically.
66. The method of claim 64, wherein said compound is administered therapeutically.
67. The method of claim 64, wherein said reperfusion injury is myocardial infarction.
68. The method of claim 64, wherein said reperfusion injury is stroke.
69. The method of claim 64, wherein said subject is a human subject.
70. The method of claim 64, wherein administering is systemic.
71. The method of claim 64, wherein administering is topical.
72. A method of improving or maintaining vascular responsiveness in a subject, the method comprising administering to a subject in need thereof an amount of an inhibitor of a poly (ADP-Ribose) polymerase (PARS) in an amount sufficient to inhibit activation of PARS in the blood vessels of said subject.
73. The method of claim 72, wherein said subject suffers from, or is at risk for, developing diabetic vascular complications.
74. The method of claim 72, wherein said subject suffers from hyperglycemia.
75. A method of identifying a candidate agent for treating diabetes, the method comprising contacting a poly (ADP-Ribose) polymerase (PARS) enzyme with a test compound; and determining whether said compound inhibits the activity of said enzyme, wherein an inhibition of said enzyme in the presence of the compound indicates said test agent is a candidate agent for treating diabetes.
76. The method of claim 75, wherein said PARS enzyme is located within an endothelial or smooth muscle cell.
77. The method of claim 76, wherein said cell is provided *in vitro*.
78. The method of claim 76, wherein said cell is provided *in vivo*.
79. A method of identifying a candidate agent for treating vascular degeneration, the method comprising contacting a poly (ADP-Ribose) polymerase (PARS) enzyme with a test compound; and determining whether said compound inhibits the activity of said enzyme,

wherein an inhibition of said enzyme in the presence of the compound indicates said test agent is a candidate agent for treating vascular degeneration.

80. The method of claim 79, wherein said PARS enzyme is located within an endothelial or smooth muscle cell.
81. The method of claim 80, wherein said cell is provided *in vitro*.
82. The method of claim 80, wherein said cell is provided *in vivo*.
83. The method of claim 79, wherein said vascular degeneration is caused by diabetes.
84. A method of enhancing the function of a transplanted organ, the method comprising:
providing said organ;

contacting said organ with an inhibitor of poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount sufficient to inhibit PARS in said organ; and

introducing said organ into a subject.
85. A method for prolonging the function of a transplanted organ in a subject, the method comprising administering to said subject a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount sufficient to inhibit PARS in said subject.
86. A method for inhibiting the development of symptoms associated with multiple sclerosis in a subject, the method comprising administering to a subject at risk for the development of said symptoms a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount to inhibit the development of symptoms of multiple sclerosis in said subject.
87. A method for treating symptoms associated with a neurodegenerative disorder in a subject, the method comprising administering to said subject a poly (ADP-Ribose) polymerase (PARS) inhibitor in an amount to alleviate symptoms of the neurodegenerative disorder in said subject.
88. The method of claim 87, wherein said neurodegenerative disorder is selected from the group consisting multiple sclerosis, AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and an encephalitis.



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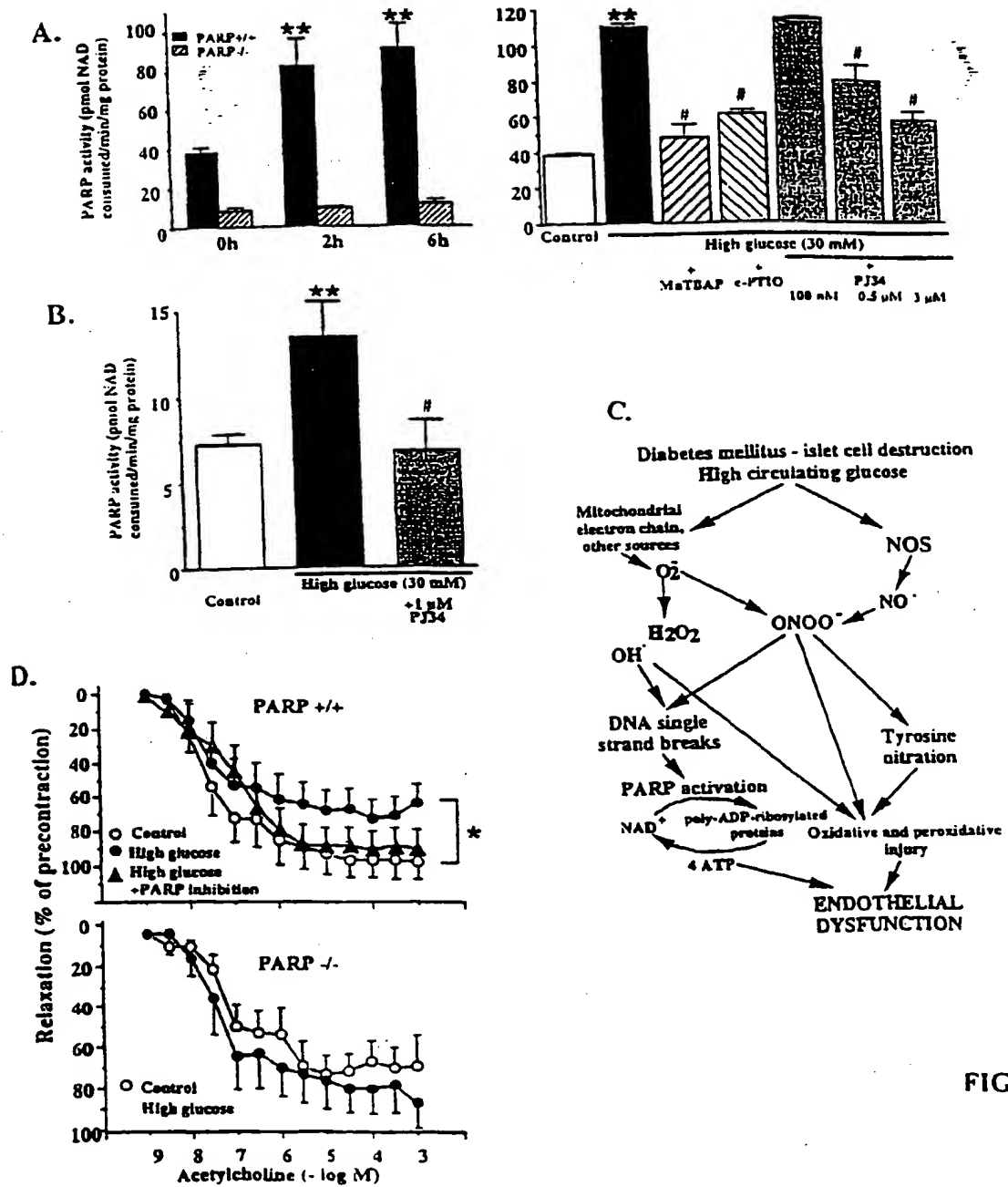


FIG 2

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